The search for and synthesis of potential antineoplastic drugs.

Webb, Brian

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A SEARCH FOR AND SYNTHESIS OF POTENTIAL ANTINEOPLASTIC DRUGS.

Submitted by BRIAN WEBB

for the degree of Ph.D of

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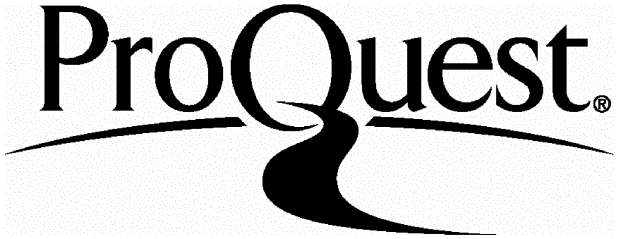

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Finally, the author's everlasting gratitude goes to his wife, Joyce and son Nicholas for their patience and understanding during the growth of this thesis.

Summary

The research described in this thesis was carried out by the author at the University of Bath between October 1971 and September 1974. The thesis is divided into three parts, the first and final parts describe phytochemical studies on two trees indigenous to the Pacific area. The remainder of the thesis is devoted to synthetic work connected with the phytochemical study.

Bleekeria vitiensis, a tree which grows in Fiji has previously been shown to be the best natural source of the alkaloid 9-methoxyellipticine. Part I of this thesis confirms this observation and also describes the isolation of holeinine and a new alkaloid, bleekerine in addition to several other extractives.

In Part II, attempts to synthesise 9-nitroellipticine are discussed. This we hoped would be a convenient intermediate for the preparation of several other derivatives which were to be tested for anticancer activity. Following this a successful preparation of 9-aminoellipticine is described.

The final section of this thesis deals with an examination of the plant Lophopetalum rigidum (Celastraceae) which it was hoped would contain macrolide bases similar to those recently isolated from sister species.

Instrumental Methods

All IR spectra were determined on a Perkin-Elmer 237 spectrophotometer as nujol mulls or liquid films. UV spectra were determined on a Perkin-Elmer 402 spectrophotometer in 95% ethanol. Early ^{1}H-n.m.r. spectra were obtained using a Varian A-60 spectrometer and later ones with a J.O.E.L. P.S. 100 spectrometer; chemical shifts are expressed in ppm downfield from tetramethylsilane as internal standard. Mass spectra were measured on an A.E.I. M.S. 12 spectrometer.

Contents

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The successful treatment of cancer in its many forms still remains one of man's most taxing problems, one potential solution, which to date has had only limited success, is the application of selective drugs. At the present time many of the anti-cancer agents in clinical use are natural products or heterocycles of various types, but little is known concerning their modes of action.

9-Methoxyellipticine (1) is an alkaloid, first isolated from Ochrosia elliptica Labil[1] and subsequently found in many sister species, which shows wide spectrum activity against mouse neoplasms. 9-Methoxyellipticine frequently co-occurs with ellipticine (2) and the first part of this thesis deals with a phytochemical investigation of a member of the Ochrosiinae - Bleekeria vitiensis A.C. Smith - which has been shown[2] to be an abundant source of these and other alkaloids.

The second part of this work describes studies directed towards the establishment of structure-activity relationships in 5,11-dimethyl--6H-pyrido[4,3-b]carbazoles (ellipticines) and thus it is appropriate in this introductory chapter to review briefly the pharmacology of these alkaloids and their derivatives and to indicate current theories on their mode of action.

In 1968 Svoboda et al[3] published the results of the first investigation of 9-methoxyellipticine as a possible neoplastic agent. It was shown that the alkaloid had significant effect against ten of the seventeen mouse neoplasms tested and further that it was active against both the ascites and solid forms of the Walker 256 rat carcinosarcoma. Le Men et al[4] have demonstrated subsequently that, as the water soluble lactate, 9-methoxyellipticine inhibits mouse

L 1210 leukemia which has a sensitivity close to that of acute human leukemias.

These encouraging results initiated clinical trials of 9-methoxy-ellipticine in France and of ellipticine in the United States of America, and recently Mathé has shown[5] the former compound to have significant activity against human myeloblastic leukemia.

The mechanism by which ellipticine inhibits the development of leukemia has been studied to some degree and it is accepted that the drug is inter-calated[6] between the base pairs of the DNA (or RNA) spiral producing a change[7] in the torsional angle of the super coiled helix.

Ellipticine shares with other inter-calating drugs such as the acridines a planar structure and DNA - ellipticine complexes have been identified[6]. Although the effect is non-specific killing cells at all stages in their growth cycle, cells at mitosis are more sensitive than S and G_2 cells[8]. Maturation of mouse lymphoma cells is primarily inhibited[9] by inter-calation of ellipticine to 45 S nucleolar RNA. Relatively few ellipticine derivatives have been prepared and tested, and in the main, these reflect ease of access rather than a selection designed to elucidate trends in biological structure relationships. It is evident, however, (see table 1) that only those structures containing the intact 5,11-dimethyl--6H-pyrido[4,3b]carbazole nucleus are likely to show useful activity.

Dalton *et al*[10] were the first to embark on the synthesis of ellipticines for anticancer screening and, using a modification of the Cranwell and Saxton synthesis[11] (page 56), a number of derivatives

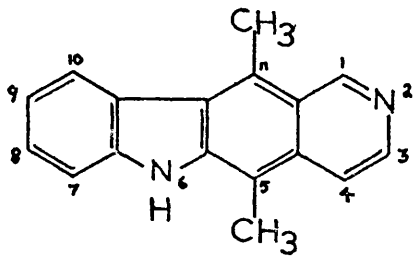
were prepared. Of these only 6-methylellipticine (3) showed activity approaching that of the two natural alkaloids. Since that time it has been suggested[12], however, that this compound is comparatively inactive a conclusion which firstly emphasises the requirement for standardised testing techniques, such as those now applied in the National Cancer Institute, N.I.H. Bethesda U.S.A., and secondly questions early pharmacological claims in the anticancer field.

Recent French work[12] indicates that 9-hydroxyellipticine (4) is rather more active than 9-methoxyellipticine (1) against L 1210 in mice, but although Rastogi et al[13] have prepared a number of 6H-pyrido [4,3b] carbazoles the results of the pharmacological analysis of these products have not been published.

It is apparent from the results summarized in table 1 that at present it is not possible to decide intuitively the best analogues to prepare. On the other hand by analysing electronic, steric and solubility characteristics among ellipticines Hantsch[15] has indicated how a judicious choice of synthetic targets can be made. These conclusions provide the stimulus for much of the work described in this thesis and our attempts to implement the synthetic challenges implicit in the Hantsch proposals are described in part II which commences with a review of past synthetic endeavour in the ellipticine field.

The third and final part in this work describes a survey of the plant Lophopetalum rigidum, a member of the Celastraceae, in the hope that it might contain a structure similar to the macrolide

Table I



(2) Ellipticine

Ellipticine derivative	P.S.*	Reference
Free base	125 (188)	12 (14)
Tartaric salt	100	12
Hydrochloride salt	115	12
9-Methoxy (1)	107 (170)	12 (14)
" " lactate	130	12
" " hydrochloride	107	12
6-Methyl (3)	108 and 100 (134)	12 (14)
9-Methyl	104 (105)	12 (14)
7,10-Dimethyl	100 (112)	12 (14)
11-Desmethyl	100 and 94 (170)	12 (14)
5,11-Desmethyl	89	12
6-Isopentyl	100	12
9-Hydroxy (4)	164	12
9-Amino	120	12
9-Bromo	108	14
9-Nitro	inactive	10
9-Methoxy, 6-methyl	152	14

* P.S. (prolongation of survival) = $\frac{\text{mean survival of treated group}}{\text{mean survival of untreated group}} \times 100$

The recent study (ref. 12) is probably a more valid estimation.

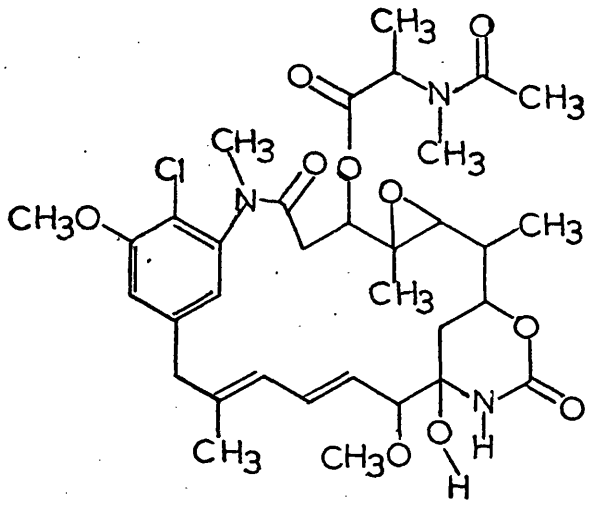
Table I (continued)

Ellipticine derivative	P.S.	Reference
(±) 1,3-Dimethyl-5,H-desmethyl 1,2,3,4-tetrahydro (±)Guatambuine	109	14
(+) Guatambuine	145≠	14
3-Methyl	105	14
1,2,3,4-Tetrahydro--9-methoxy-2-methyl	102	14
1,5-Dimethyl (olivacine)	151	14
3,4-Dihydro-1,5-dimethyl	123	14
Isoellipticine**	113	14
3,4-Dihydroisoellipticine**	110	14

** Basic N at position 3

≠ Not reproducible

maytansine (5) which is found[16] in related species. Maytansine is one of the most active anticancer agents known[17].



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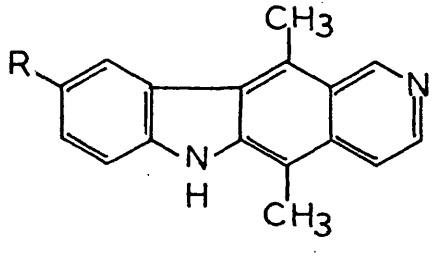
PART 1

PHYTOCHEMICAL ANALYSIS OF BLEEKERIA VITIENSIS

In 1969 an examination[2] of the Apocynaceous plant Bleekeria vitiensis (Markgraf) A.C. Smith, sub-family Ochrosiinae was undertaken in this department in order to evaluate it as a source of the potentially valuable alkaloid 9-methoxyellipticine (1). This alkaloid was known to be present in other members of the sub-family. Table II summarizes the alkaloid content of the dozen or so species which have been studied to date.

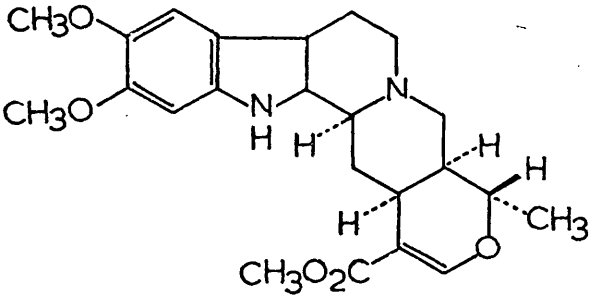
A systematic search of the leaves and leaf stems, combined bark and trunk wood and also the roots of Bleekeria vitiensis revealed the presence of several compounds (see Fig. 1). Moreover, the bark and trunk wood was found to be the richest natural source of 9-methoxyellipticine known at present.

Fig. 1.

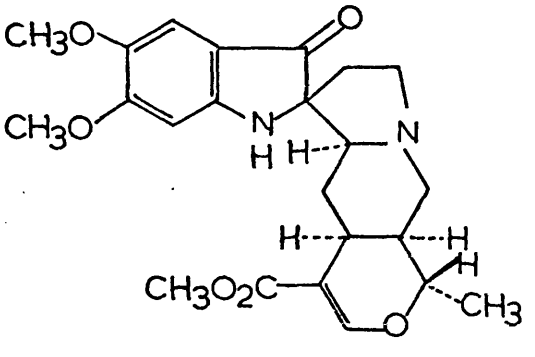


(1) R=OMe 9-methoxyellipticine

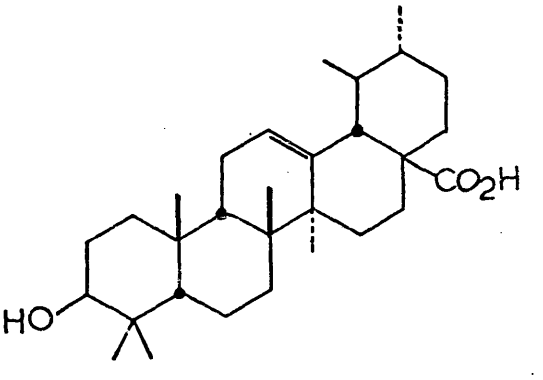
(2) R=H ellipticine



(6) isoreserpiline



(7) isoreserpiline-ψ-indoxyl



(8) ursolic acid

Table II

Alkaloid Content of the Ochrosia

Plant and Source	Part of Plant	Alkaloids	Reference
Ochrosia elliptica Labill. Florida U.S.A.	leaves and leaf stems	Ellipticine(2) 9-methoxy-ellipticine (1),isoreserpiline (6),elliptinine ($C_{20}H_{24}O_2N_2$)	1, 18
Ochrosia moorei F. Muel. Australia (these are specimens of O.elliptica transplanted originally from Trinidad)	leaves and leaf stems	Isoreserpiline(6) elliptamine ($C_{24}H_{30}O_5N_2$)	19
Ochrosia coccinea (Tejsmann and Binnendijk) Miq. (syn. Excavatia coccinea (Tejsmann and Binnendijk)Mgk. New Guinea	Bark and leaves	9-Methoxyellipticine (1), reserpine, elliptamine	18, 19, 20
Ochrosia sandwicensis (ADC) A.Gray (syn. Bleekeria calocarpa) Hawaii	Leaves trunk and root bark	(a) Ellipticine (2) (t, 0.04%) 9,methoxyellipticine (1) (b) 10-Hunterburnine--methochloride (t, 0.07%) 10-hydroxydihydro--corynantheol metho--chloride (c) Holeinine	(a) 21, (b) 21 (c) 22
Ochrosia malculata Jacq. (syn. Ochrosia borbonica Gmel. or Cerbera undulata. Reunion Island.	Bark	9-Methoxy--ellipticine (1) (0.015%) reserpine	23, 24, 25
Ochrosia poweri Bailey, Queensland Australia.	Leaves leaf stems and twigs	(a) Isoreserpiline (6), 0.3% in stems, reserpine, powerine, ($C_{21}H_{26}N_2O_4$), Poweridine($C_{24}H_{30}N_2O_5$), poweramine($C_{23}H_{30}N_2O_4$) (b) Elliptamine (c) Ochropamine ($C_{22}H_{26}N_2O_3$), ochropine($C_{23}H_{25}N_2O_4$), powerchrine($C_{22}H_{26}N_2O_3$)	(a) 19 (b)19,26 (c) 26

Table II (continued)

Plant and Source	Part of Plant	Alkaloids	Reference
Ochrosia oppositifolia (Lamk.) K. Schum. Mascarene and Seychelle Islands	Bark	(a) 9-methoxyelliptioine (1) (b) Reserpiline, isoreserpiline (6), 10-methoxydihydrocory--nantheol, ochrolifuanine A., reserpinine, isoreserpinine ochropposine and ochropposinine	(a) 27 (b) 28
Ochrosia vieillardii Guill. New Caledonia	leaves and bark	(a) Ellipticine (2) 0.0079%, isoreserpiline (6) 0.085% 10-methoxydihydro-corynantheol (b) Reserpiline, dimethoxypicraphylline Ochropposinine, 1,2-dihydroellipticine, 1,2,3,4-tetrahydro--ellipticine	(a) 29 (b) 30
Ochrosia glomerate Valeton, New Guinea	Leaves	Isoreserpiline (6), elliptamine	19
Ochrosia silvatia Dan. New Caledonia	Trunk bark.	Ellipticine (2) 0.13%, isoreserpiline (6) 0.58%, apparicine 0.012%	31
Ochrosia balansae Baill. ex. Guill. (syn. Excavatia balansae Guill.) New Caledonia	Leaves and bark	(a) Ellipticine (2), isoreserpiline (6), aricine, reserpiline (b) Dimethoxypicraphylline ($C_{24}H_{30}N_2O_4$) 1,2-Dihydro-9-methoxy--ellipticine, 1,2,3,4-tetrahydro--ellipticine and 1,2,3,4-tetrahydro-9--methoxyellipticine	(a) 32 (b) 33

Table II (continued)

Plant and source	Part of Plant	Alkaloids	Reference
Ochrosia lifuana Guill. New Caledonia	Leaves	(a) Ochrolifuanine (A and B)($C_{29}H_{29}N_3$)	(a) 34
		(b) Dehydro-3-ochroli--fuanine ochrolifuanine A or B --N_b-oxide	(b) 35
		(c) Decarbomethoxy--dihydro-gambitrannine ($C_{19}H_{18}N_2$)	(c) 36
Ochrosia confusa Pichon. New Caledonia	Trunk bark	Ellipticine, rauvoxine, carapanaubine, ochrolifuanine, dehydro-ochrolifuanine and 10-methoxydihydro -corynantheol	37
Ochrosia miana H. Bn. ex Guill. New Caledonia	Leaves	Decarbomethoxydihydro--gambitrannine	36
Bleekeria vitiensis (Markgraf) A.C.Smith syn. Ochrosia vitiensis or Excavatia vitiensis Fiji.	Leaves and leaf stems, Trunk and roots	9-Methoxyellipticine (l, 0.003%; t, 0.083%; r, 0.024%) ellipticine (t, 0.001%; r, 0.005%) isoreserpiline (l, 0.05%; t, 0.002%; r, trace). Isoreserpiline-ψ--indoxyl(7) (l, 0.001%; t, trace; r, trace)	2

* l = leaf and leaf stems,

t = bark and trunk wood,

r = root and root bark.

To establish the generality of this high level of 9-methoxy-ellipticine in B.vitiensis a fresh sample of this plant has been investigated. For this study, however, the bark and trunk wood were examined separately so that the alkaloid content of each could be estimated.

General procedures

Bark

The finely divided dry stem bark (500g) was exhaustively extracted with hot ethanol. Evaporation of the solvent under reduced pressure furnished a brown gum which when chromatographed over alumina, eluting with petrol (60-80)-chloroform, chloroform and chloroform-methanol mixtures, afforded three main fractions A, B and C.

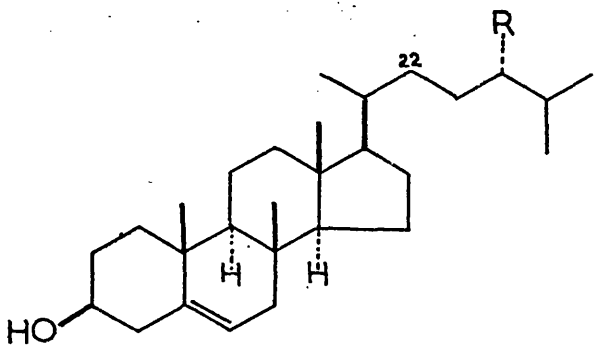
Fraction A.

It appeared from thin layer chromatographic analysis that this material contained three major components and these were separated by further chromatography over alumina.

The first fractions showing only one spot on TLC (Al_2O_3) were combined and evaporated to give an off-white solid which was crystallized from aqueous methanol. Precision mass spectrometry showed this material to be a mixture of three compounds of molecular weights 414.3844 ($C_{29}H_{50}O$), 412.3705 ($C_{29}H_{48}O$) and 400.3705 ($C_{28}H_{48}O$).

The occurrence of natural products with molecular formulae of this order is suggestive of sterols and from systematic tables of mass spectrometric data we considered β-sitosterol (9), campesterol (10) and stigmasterol (11) to be the most likely

candidates for these compounds. Our suspicions were confirmed when a mixture of these three sterols gave an identical mass spectrum to that of the extractive.



(9) R = C_2H_5

(10) R = CH_3

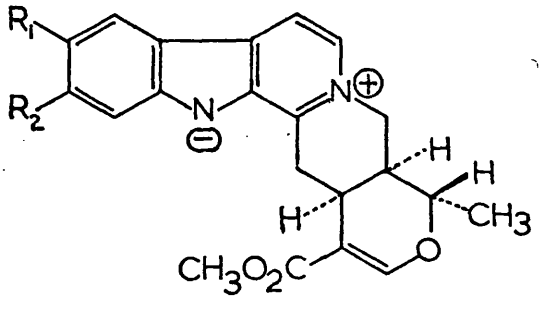
(11) R = C_2H_5, Δ^{22}

Further chromatography of fraction A failed to provide distinct components and the remaining sub fractions were combined, and on evaporation, these gave a mixture of isoreserpiline (6) together with a smaller proportion of isoreserpiline-ψ-indoxyl (7).

Fraction B.

A thin layer chromatograph (Al_2O_3) of this gummy extract when viewed under ultraviolet light displayed a highly fluorescent blue-green spot. Subsequent trituration of this material with methanol caused crystallization and afforded a yellow solid which recrystallized

from ethanol in the form of prisms, m.p. 276-7°. This compound had not been found in the previous study and in fact it was shown to be a new alkaloid to which the name "Bleekerine" has been given. Assignment of structure (12, $R_1 = R_2 = OMe$) was made from spectral data (see page 22) and synthetic studies. Thus sodium borohydride reduction of bleekerine provided a compound identical to isoreserpiline (6) in all respects. Furthermore when oxidized with $Pb(OAc)_4$ isoreserpiline gave a mixture of bleekerine together with isoreserpiline-γ-indoxyl(7)[38].



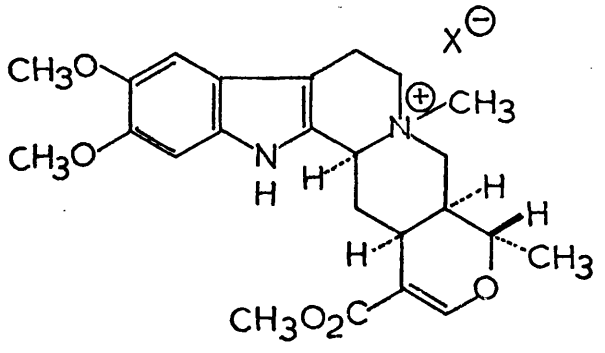
(12)

Crude bleekerine contains traces of another compound which, in the mass spectrometer, gives rise to a molecular ion m/e 422.1842 ($C_{24}H_{26}N_2O_5$) together with ions due to methyl chloride and hydrogen chloride. Possibly this molecule is N(a)-Methyl--bleekerine.

The remaining portion of the fraction from which bleekerine was obtained was triturated with ether and filtered to give a yellow-brown solid. This solid was largely water soluble and was suspected

to be of similar constitution to a complex mixture of quaternary salts which had previously been obtained from the first sample of Bleekeria vitiensis. Purification attempts in the former investigation were terminated when the researcher experienced ill-effects, and subsequent pharmacological testing showed the salts to be CNS active.

However, on treatment of an aqueous solution of this crude material with perchloric acid a tan coloured precipitate was formed. Crystallization from water then gave a small quantity of holeinine perchlorate (13, X = ClO_4) (this conclusion is supported by infrared and melting point comparisons with an authentic sample[22]). This alkaloid was also obtained from fraction C, but this time as mixed chloride/bromide salts.



(13)

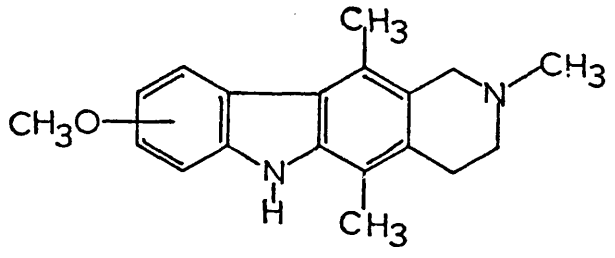
Fraction C.

9-Methoxyellipticine (1) which has limited solubility in most solvents crystallized out when this fraction was partially evaporated, and recrystallization from ethyl acetate gave several crops of the pure alkaloid. From the mother-liquors a mixture of 9-methoxy-ellipticine and ellipticine was obtained. Partial purification of this mixture was achieved by crystallization from benzene, giving elliptioine (2) (13mg) contaminated with 9-methoxyellipticine (1).

In the original investigation of B.vitiensis, ellipticine was obtained free from 9-methoxyellipticine by preparative layer chromatography and repeated crystallizations, therefore, in view of the small quantity of material available, further purification was not attempted.

Methoxyellipticine was first isolated[1] in 1959 but the correct structure remained uncertain for several years. Woodward[39] had proved ellipticine to be 5,11-dimethyl-6H-pyrido [4,3b] carbazole soon after it was isolated and it was clear from the ultraviolet absorption spectrum of methoxyellipticine that the same heteroaromatic ring system was present. Jordan and Scheuer[21] by comparing the ultraviolet and fluorescence spectra of a sample of the alkaloid isolated from Ochrosia sandwicensis with those of 5- and 6-methoxy-indoles suggested it was 8-methoxyellipticine. However, Loder[18] later pointed out that while it was reasonable to assume that the methoxy group occurs in ring A of the pyrido-carbazole nucleus , the position of the group could not be assigned with certainty.

In 1966 Loder[18] reduced methoxyellipticine methiodide to the tetrahydro derivative (14) and from detailed analysis of the nuclear magnetic resonance spectrum he was able to deduce the correct structure i.e. the methoxy group is allocated to position - 9. Final proof was obtained by synthesis from 5-methoxyindole using a modification of Cranwell and Saxton's route[11] to ellipticine.



(14)

The remaining material of Fraction C, now almost free of 9-methoxy ellipticine was subjected to further chromatography (Al_2O_3). Eluting first with increasing proportions of chloroform in petrol (60-80°) and then methanol in chloroform, four major sub-fractions (D, E, F and G) were acquired.

Fraction D (10mg) was recognized from the mass spectrum to be a crude mixture of the sterols previously identified.

From TLC analysis fractions E and G were observed to be virtually identical each showing blue-green and red fluorescent spots under soft ultra-violet light characteristic of ellipticine and 9-methoxyellipticine respectively.

Trituration of fraction F with petrol and then hot dichloroethane furnished a yellow solid. An aqueous solution of this product, when filtered, evaporated and the residue crystallized from chloroform gave a colourless crystalline solid (75mg). Spectral data of this compound showed it was mixed chloride/bromide of holeinine.

(13, X = Cl^-/Br^-). The presence of a molecular ion m/e 440.2310 ($C_{25}H_{32}N_2O_5$) is observed in the mass spectrum of holeinine (Fig. 2) and it is significant that the same ion is also present in the mass spectrum of a sample of holeinine obtained from O. sandwicensis. It is possible that the origin of this molecular ion is the, as yet unreported, alkaloid salt N(a)-methylholeinine, but because of the extremely low concentration in the plant it has not been possible to confirm this.

Wood

Extraction of the heartwood of Bleekeria vitiensis with ethanol gave a relatively low yield of product (0.113g from 500g) chromatographic analysis of which showed it to comprise ellipticine, 9-methoxyellipticine (1) isoreserpiline (6) isoreserpiline-ψ-indoxyl (7) and the sterols β-sitosterol (9) stigmasterol (11) and campesterol (10). These components were partially separated by preparative layer chromatography.

The results of the above study are summarized in table III.

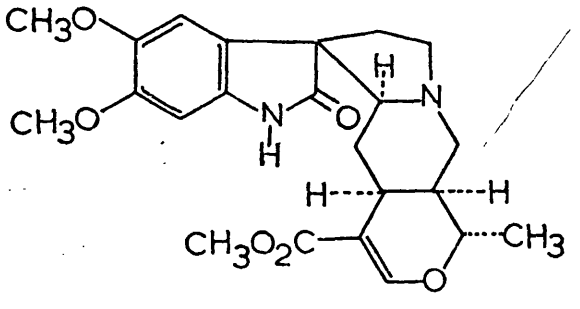
Table III

Part of Plant	Compound	% Yield
Bark	β-Sitosterol	
	Stigmasterol	0.011
	Campesterol	
	Isoreserpiline	0.01*
	Isoreserpiline-ψ-indoxyl	trace
	Bleekerine	0.009
	Holeinine (Cl /Br)	0.016
	9-Methoxyellipticine	0.165
	Ellipticine	0.002*
Wood	β-Sitosterol	
	Stigmasterol	0.001
	Campesterol	
	Isoreserpiline	Trace
	Isoreserpiline-ψ-indoxyl	Trace
	9-Methoxy-ellipticine	0.006
	Ellipticine	Trace

* Approximate

In view of the interesting results of this investigation a third study[40] of Bleekeria vitiensis was commenced in this laboratory. The reason for this was two-fold, firstly to obtain sufficient bleekerine for pharmacologicaltesting and also to try and characterize the unidentified compounds detected in mass spectral studies of previous extracts.

Unfortunately, neither bleekerine nor compounds corresponding to molecular ions m/e 422 and m/e 440 were found in this sample. However, the alkaloid carapanaubine (15) was isolated.



(15)

This result emphasises the need for great care in the collection of botanical specimens since there is certainly a seasonal variation in alkaloidal constituents within plants and probably also a change from site to site even within a small area of habitat. Unfortunately, precise details of collection dates for our specimens were not available to us.

Physical Data of Extractives

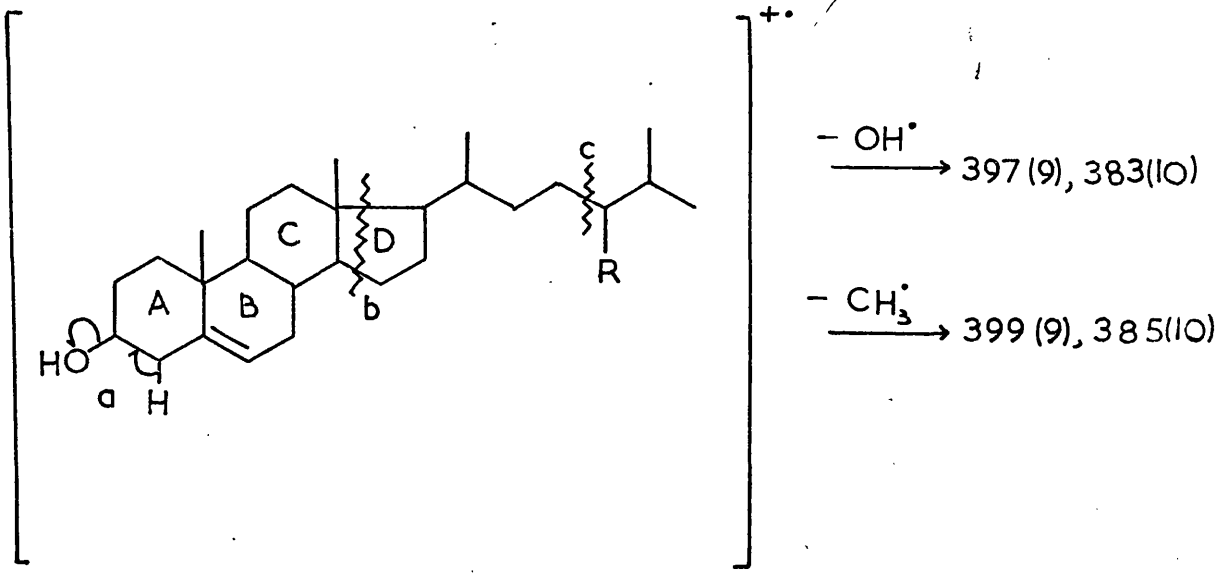
Sterols

β-Sitosterol (9) stigmasterol (11) and campesterol (10), as previously mentioned were isolated from Bleekeria vitiensis as a mixture and their identification rests upon mass spectral comparison with authentic specimens and from gas-liquid chromatographic (GLC) data.

The mass spectrum (Fig. 3) of the mixed sterols is complex but an analysis of the main features of the spectrum is provided in the following scheme:

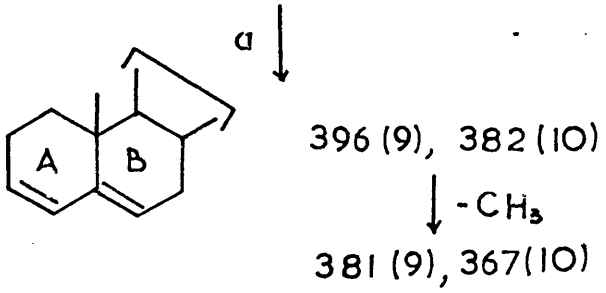
M.S.I.

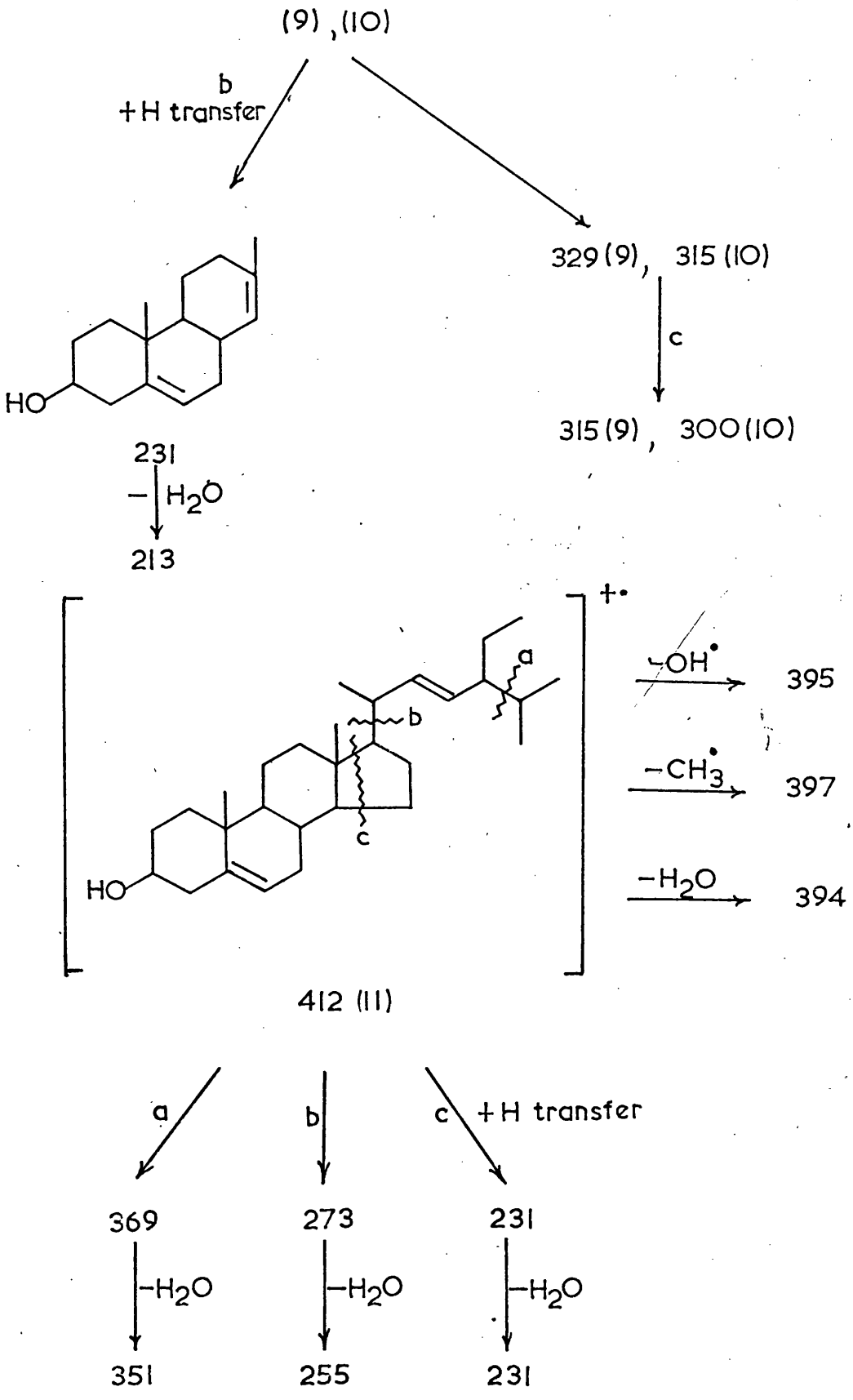
Fragmentation Pattern of β-Sitosterol, Stigmasterol and Campesterol.



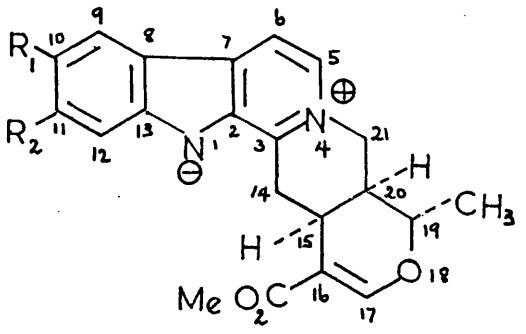
(9) R = C_2H_5 414

(10) R = CH_3 400




(9), (10)
b
+H transfer
329 (9), 315 (10)
c
315 (9), 300 (10)
HO
231
−H2O
213
+•
a
b
c
HO
−OH•
395
−CH3•
397
−H2O
394
412 (11)
a
b
c
+H transfer
369
273
231
−H2O
−H2O
−H2O
351
255
231

Spectral Characteristics of Bleekerine (12, $R_1 = R_2 = OMe$)



(12)

The electronic absorption spectrum (Fig. 4) of bleekerine suggested a highly conjugated structure ($\lambda_{max}(\varepsilon)$: 208 (14,000), 242 (14,900), 276 (9850), 336 (12,150) and 394 (5,600) nm) while the infra red spectrum (Fig. 5) demonstrated a similarity to iso-reserpiline, with bands at 2660, 1695, 1630, 1625 and 1215cm^{-1}. Significantly, however, the band anticipated for the N-H group of the indole unit was absent and an additional band at 1630cm^{-1} was observed; this last band was ascribed to the presence of a $-C=\overset{+}{N}$ group.

In the mass spectrum (Fig. 6), apart from an M^+-15 ion (80%), very little fragmentation was observed, the molecular ion being also the base peak. This fact confirms the conjugated nature of the molecule and at this point we suspected that a structure analogous to alstonine (12, $R_1=R_2=H$) might be present since the mass spectrum of this substance, kindly donated by Professor E. Wenkert, was very similar to that of bleekerine.

The ^1H n.m.r. spectrum (Fig. 7) of the alkaloid is summarized below and confirms that bleekerine is 10,11-dimethoxyalstonine, further proof was obtained by synthetic studies (page 52).

1.56 p.p.m., 3H, d ($\underline{J}$ = 6Hz) CH - $\underline{CH_3}$

2.4 - 4.6 p.p.m., 6H, multiplet aliphatic protons

3.93 p.p.m., 3H, S } - $CO_2\underline{CH_3}$ and

4.12 p.p.m., 6H, S } 2 x -$\underline{OCH_3}$

4.92 p.p.m., 1H, 2 x d ($\underline{J}$ = 14.0, 7.3Hz); C_{21} - $\underline{H}^{*}_{(a)}$

7.30 p.p.m., 1H, S }

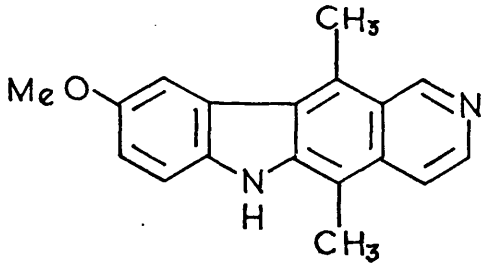
7.70 p.p.m., 1H, S, } C_9 - $\underline{H}$, C_{12} - $\underline{H}$

7.95 p.p.m., 1H, S, } C_{17} - $\underline{H}$

8.20 p.p.m., 2H, S, C_5 - $\underline{H}$, C_6 - $\underline{H}$

* The signal due to C_{21} - $\underline{H}$ (β) is superimposed upon that of C_{19} - $\underline{H}$ at ~ 4.6 p.p.m.

Spectral Characteristics of 9-Methoxyellipticine (1)



(1)

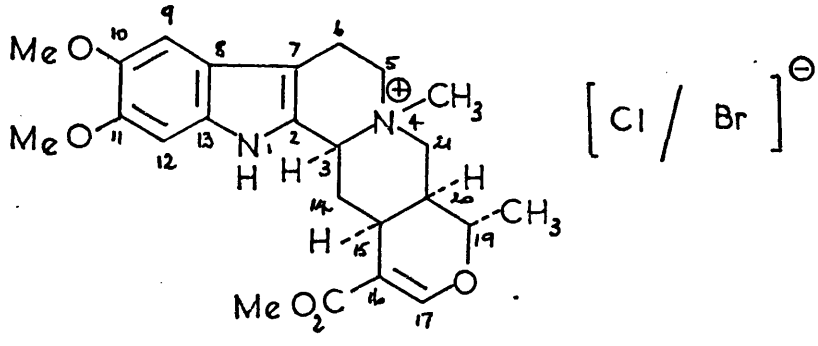
The electronic absorption spectrum of 9-methoxyellipticine (Fig. 8) is characteristic of the 6$\underline{H}$-pyrido[4,3-b]carbazole skeleton and exhibits the following maxima:- λ_{max}, (ε)245 (25,000), 278 (43,400), 295 (51,000), 307 sh. (29,600) and 357 (3,400)nm. Infrared maxima

occur at 1625, 1605 and 1495 (Ar) and 3,200 (N-H)cm^{-1}. The ^1H n.m.r. spectrum (DMSO) is reproduced in Fig. 9.

2.75 p.p.m.,	3H,	S,	$C_5 - \underline{CH_3}$
3.20 p.p.m.,	3H,	S,	$C_{11} - \underline{CH_3}$
7.15 p.p.m.,	1H, doublet of doublets ($\underline{J}$ = 9Hz and 2Hz)		$C_8 - \underline{H}$.
7.45 p.p.m.,	1H,	d, ($\underline{J}$ = 9Hz),	$C_7 - \underline{H}$
7.85 p.p.m.,	1H,	d, ($\underline{J}$ = 2Hz),	$C_{10} - \underline{H}$
7.8–7.9 p.p.m.,	1H,	m,	$C_4 - \underline{H}$
8.25 - 8.45 p.p.m.,	1H,	m,	$C_3 - \underline{H}$
9.55 - 9.75 p.p.m.,	1H,	m,	$C_1 - \underline{H}$

The simplicity of the mass spectrum (Fig. 10) of 9-methoxyellipticine reflects the stability of the molecule. The parent ion is also the molecular ion (m/e 276) and the fragmentation pattern stems from the loss of extra-skeletal units. The half mass ion (m/e 138) is prominent in the spectrum.

Spectral characteristics of Holeinine (Cl^-/Br^-) (13)



(13)

The ultraviolet spectrum of holeinine is almost identical to that of isoreserpiline hydrochloride and shows the following maxima: λ_{max}, (ε), 224 (60,560), 298 (11,500), 304 (11,350) and 309 (10,720)nm.

Characteristic absorptions in the infrared spectrum (Fig. 11) which have previously been assigned to the β-alkoxy acrylic ester system occur at 1695 and 1634cm^{-1}.

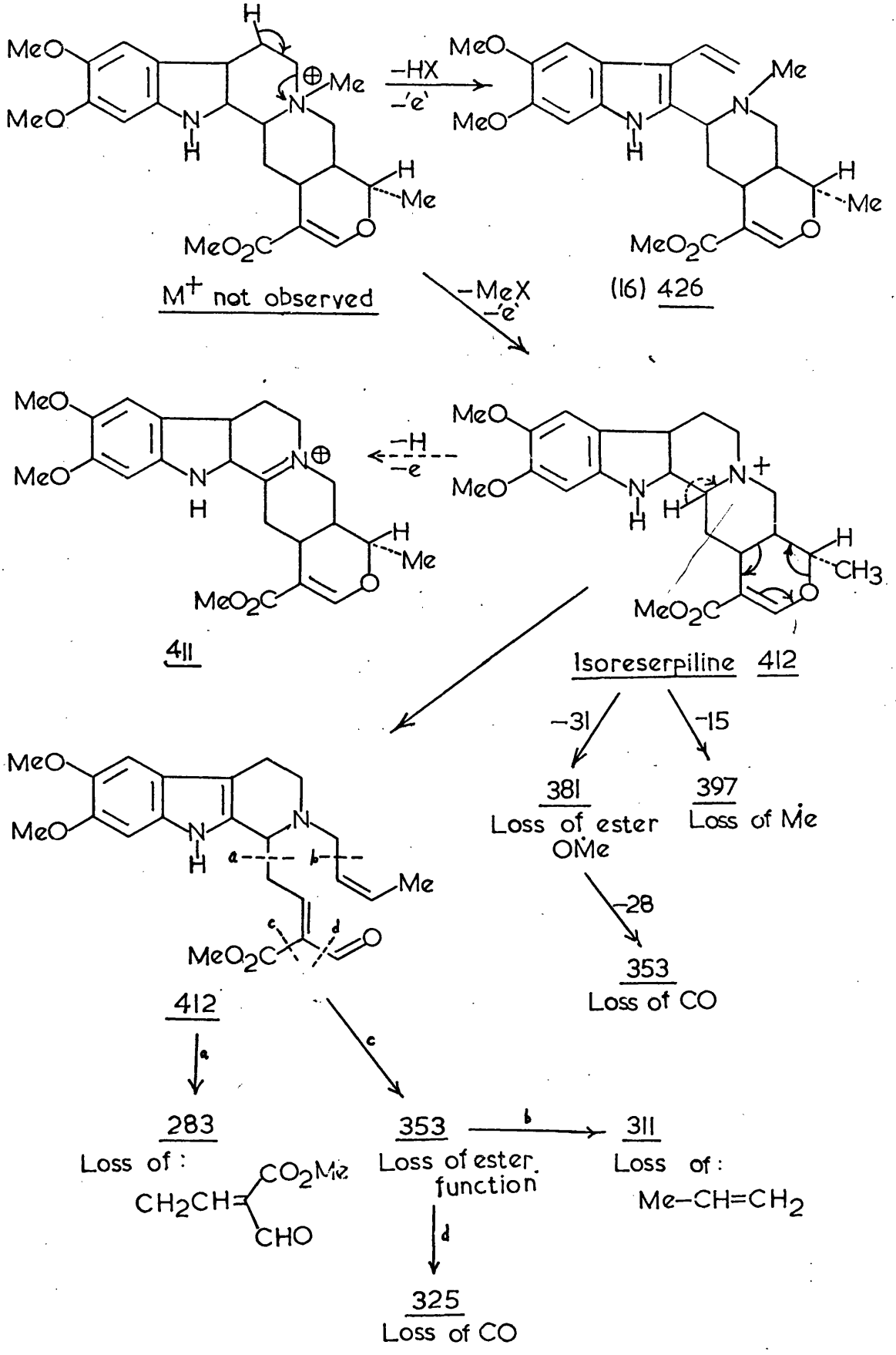
The ^{1}H n.m.r. spectrum is reproduced in a tabular form below:

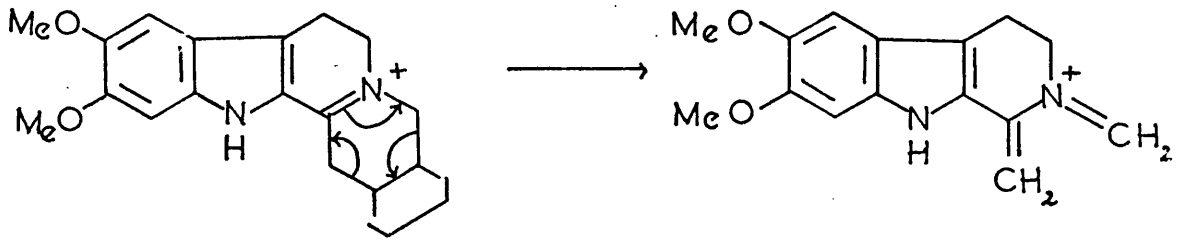
1.52 p.p.m.,	3H,	d (J = 6Hz)	C_{19} - $\underline{CH_3}$
2.70–2.40 p.p.m.,	3H,	complex,	C_{14} - $\underline{2H}$, C_{20} - $\underline{H}$
3.35–3.10 p.p.m.,	3H,	complex,	C_6-$\underline{2H}$, C_{15} - $\underline{H}$
3.50 p.p.m.,	3H,	S,	$COO\underline{CH_3}$
3.85 p.p.m.,	6H,	S,	2 x Ar - $O\underline{CH_3}$ } superimposed
4.1–3.5 p.p.m.,	8H	complex	C_3-$\underline{H}$, C_5-$\underline{2H}$, C_{21}-$\underline{2H}$, N-$\underline{CH_3}$ }
4.6–4.3 p.p.m.,	1H,	multiplet	C_{19} - $\underline{H}$
7.15–7.05 p.p.m.,	1H,	broad singlet,	C_9-$\underline{H}$, C_{12} - $\underline{H}$
7.60 p.p.m.,	1H,	S,	C_{17} - $\underline{H}$

On pyrolysis holeinine forms isoreserpiline[22], we have observed that this reaction is replicated in the mass spectrometer (see Fig. 2) giving rise to isoreserpiline and the appropriate methyl halide. Moreover, a Hofmann type elimination also occurs, affording the molecular species (16) or its equivalent, plus hydrogen chloride and hydrogen bromide. An interpretation of the spectrum is shown as follows:

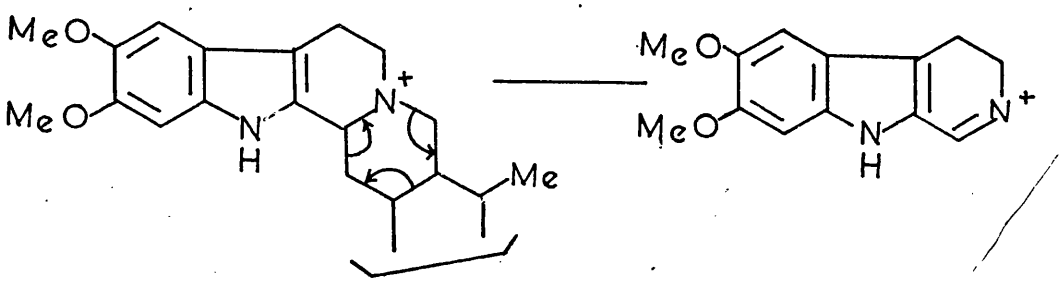
M.S. 2

Fragmentation Pattern of Holeinine

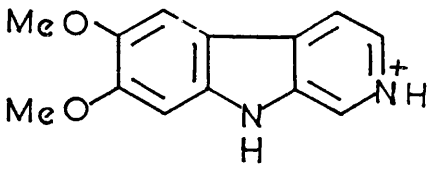




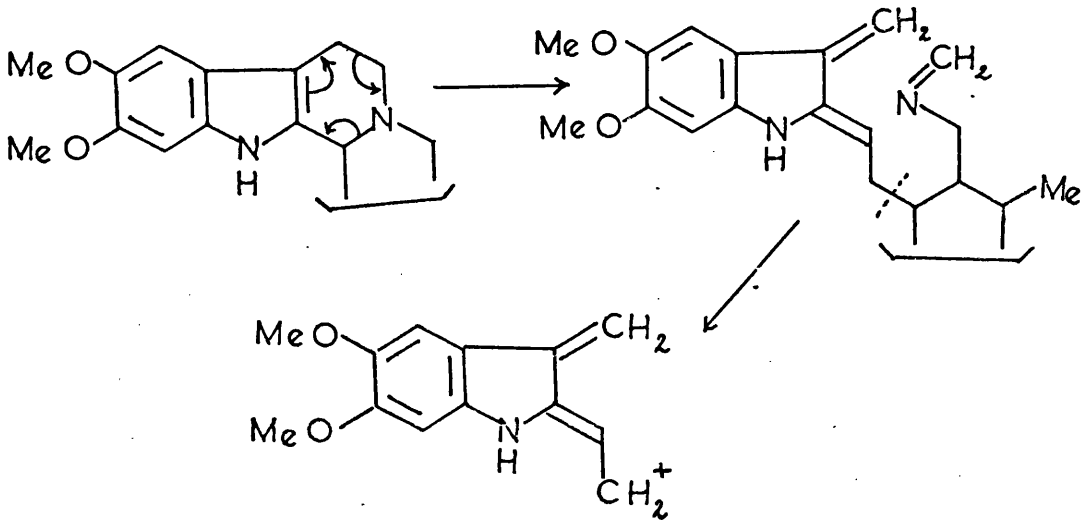
411 257



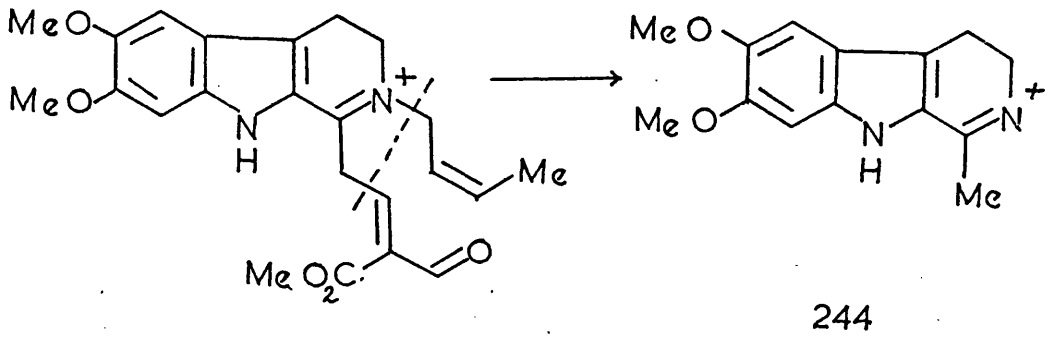
412 230



229



216

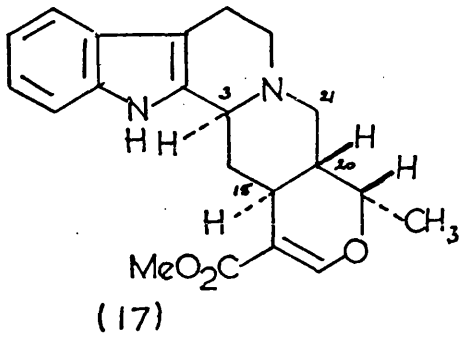


244

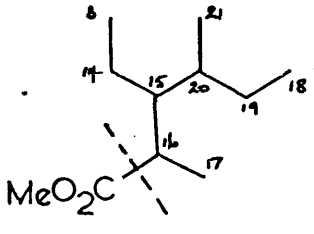
Biosynthesis

(i) The Terpenoid Indole Alkaloids

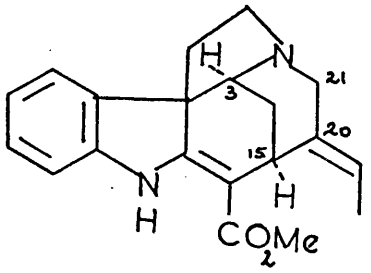
The several hundreds of known alkaloids in this group are now generally recognized to fall into three main catagories: the Corynanthe-Strychnos type e.g. ajmalicine (17) and akuammicine (18) in which the skeletal unit (19) is apparent, the Aspidosperma type e.g. vindoline (20) containing the unit (21) and the Iboga type e.g. catharanthine (22) having the arrangement (23).



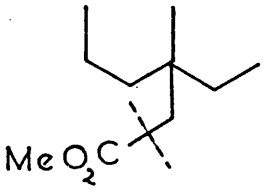
(17)



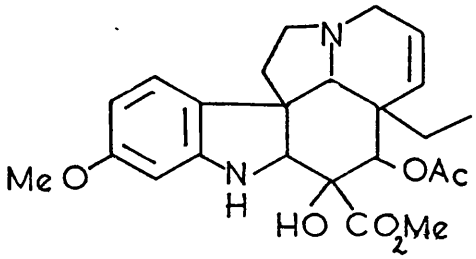
(19)



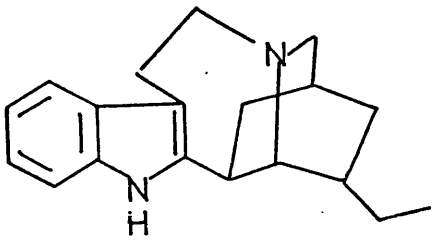
(18)



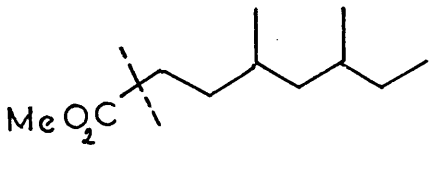
(21)



(20)

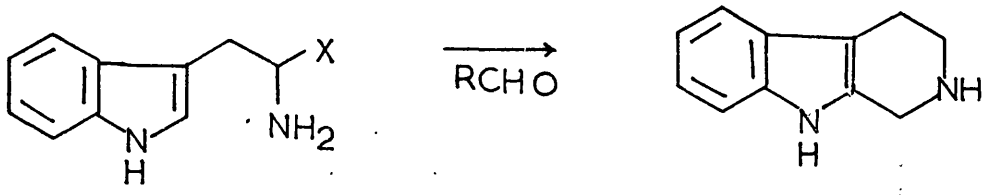


(22)



(23)

The *in vitro* formation of tetrahydro-β-carbolines (24) by a Mannich-type condensation involving tryptamine (25, X = H) and an aldehyde has been known[41] for some time and recent work has shown that tryptamine derived *in vivo* from tryptophan (25, X = CO_2H) is an effective precursor of the alkaloids.[42a,b. 43]



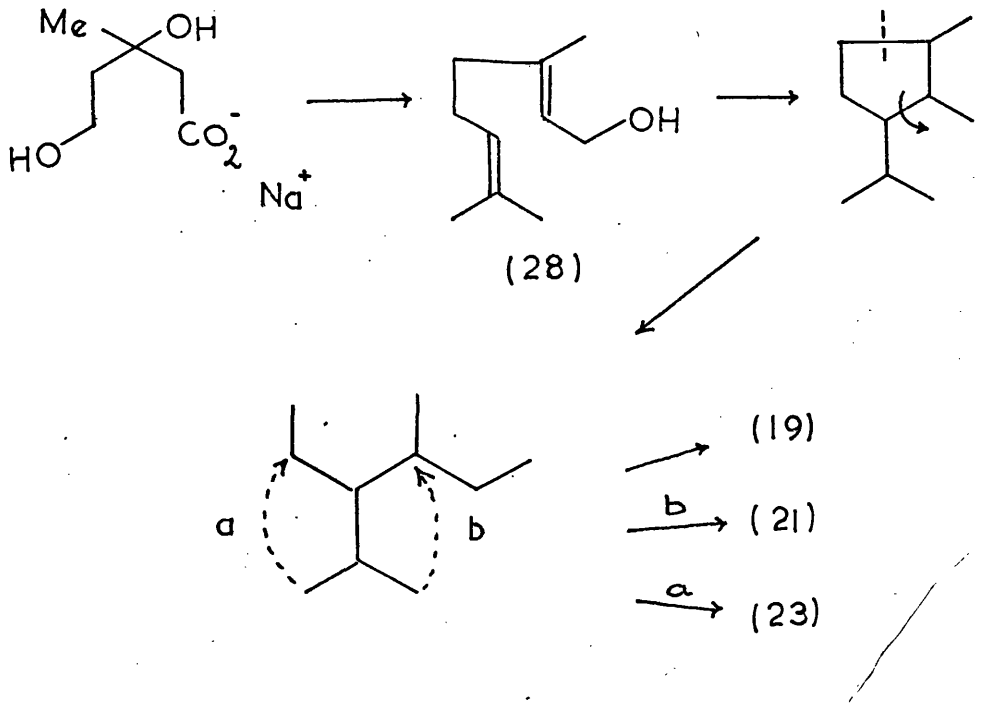
(25) (24)

The non-tryptamine portion of these molecules may consist of a ten or (when the carbomethoxy carbon atom is missing) a nine carbon atom unit.

In the early 1960's Thomas[44] and Wenkert[45] postulated units (19, 21 and 23) to originate from the monoterpenoid skeleton (26) in the manner indicated in Scheme 1. This hypothesis was substantiated some years later when feeding experiments[46] with sodium 2-[^{14}C] mevalonate in various plants produced specifically labelled alkaloids representing each of the three major types.

These results provided good evidence that the normal head-to-tail joining of two mevalonate residues with subsequent rearrangements occurred as shown in Scheme 1, though at which stage the units (21) and (23) were generated remains uncertain.

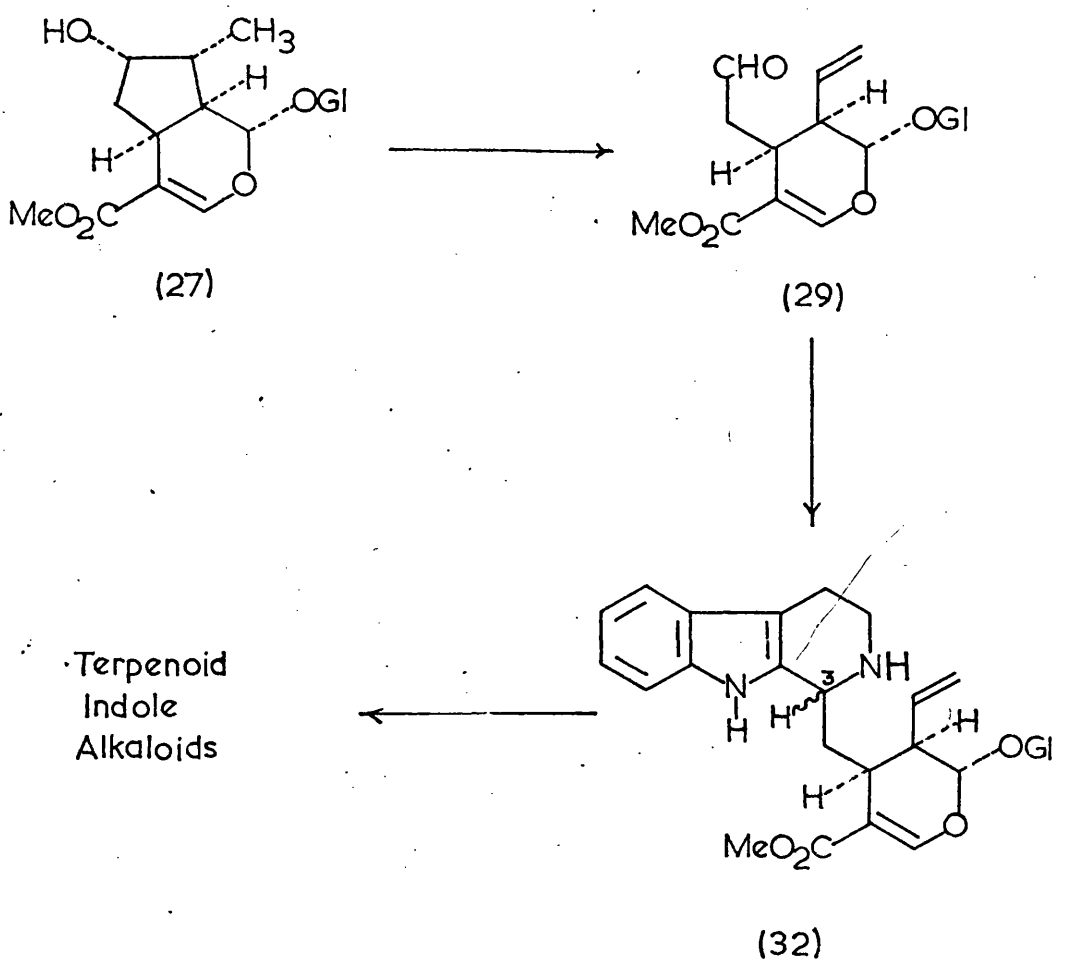
Scheme 1.



Furthermore the cyclopentane monoterpene actually involved in the biosynthetic route was identified as loganin* (27) and the part-synthesis represented in Scheme 2 was proposed.

* The stereochemistry of loganin (26) was established later by optical correlations, n.m.r. spectroscopy and x-ray analysis of a simple derivative.

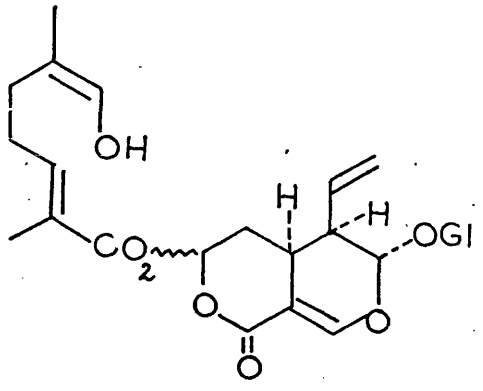
Scheme 2



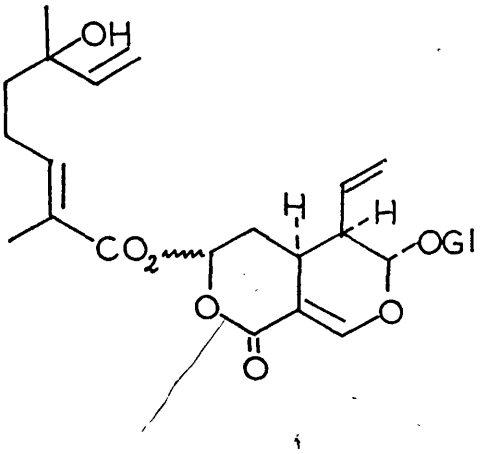
Gl = Glucose

Final proof that loganin (27) was the key intermediate was obtained when labelled loganin, prepared biosynthetically from both 2 - [^{14}C]and 4 - [^{14}C]geraniol (28) was incorporated by Vinca rosea to give specifically labelled alkaloids: ajmalicine, (17) vindoline (20) and catharanthine (22).[47,48]

The intermediacy of secologanin (29) was established[49] soon after the isolation of the terpenoids foliamenthin (30) and menthiafolin (31)[50,51]; at this point O-methyl-[^{3}H]secologanin became available for feeding experiments. Secologanin (29) itself has recently been isolated as a natural product[52].



(30)



(31)

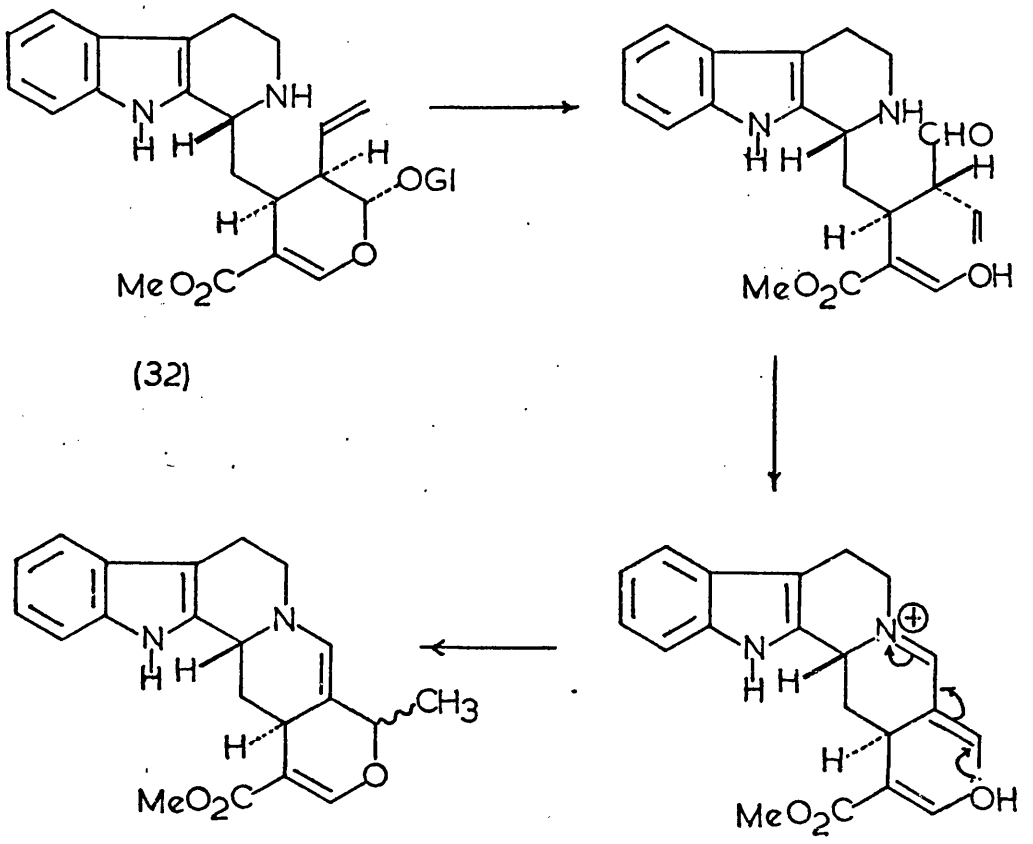
In 1968 the evidence for what could be called the first phase of terpenoid indole alkaloid biosynthesis was complete; thus isotopically labelled vincoside (32)* and isovincoside (32)* were isolated from Vinca rosea[42a,b] plants which had been 'fed' with 5-[^{3}H] loganin. Vincoside and isovincoside differ only in the stereochemistry of the 'C_3' position and, importantly, it was shown that isovincoside was not incorporated into any of the three types of alkaloid[53].

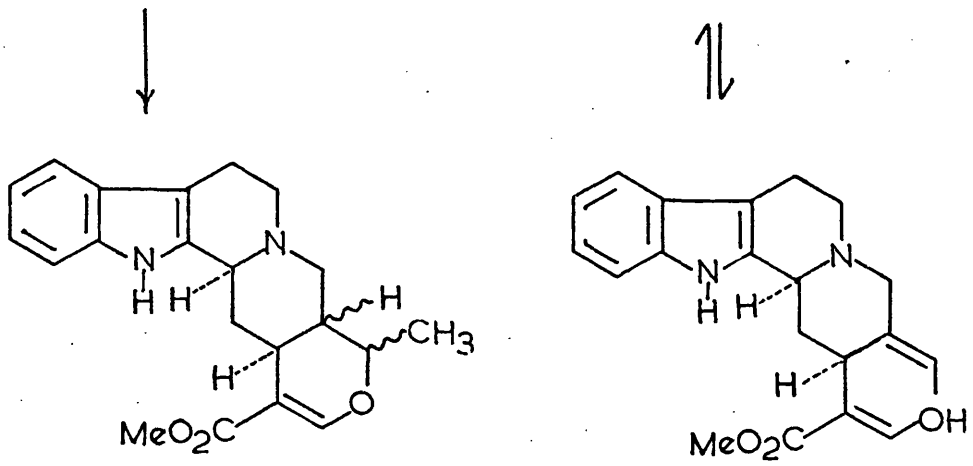
* At this time the orientation of C_3-H was unknown.

Surprisingly, the C-3 proton in vincoside has recently been identified[54,55] as β-orientated which is opposite to that of an immediate bio-derivative geissoschizine (33). The epimerization of this site undoubtedly does not involve carbon-hydrogen bond fission since the incorporation[53] of loganin C_5-hydrogen into each of the three alkaloid types has been confirmed.

The foregoing results prove that the main skeleton of vincoside is incorporated intact into the Corynanthe, Aspidosperma and Iboga systems and the formation of the Corynanthe system from vincoside (32) follows as shown in Scheme 3[42b].

Scheme 3



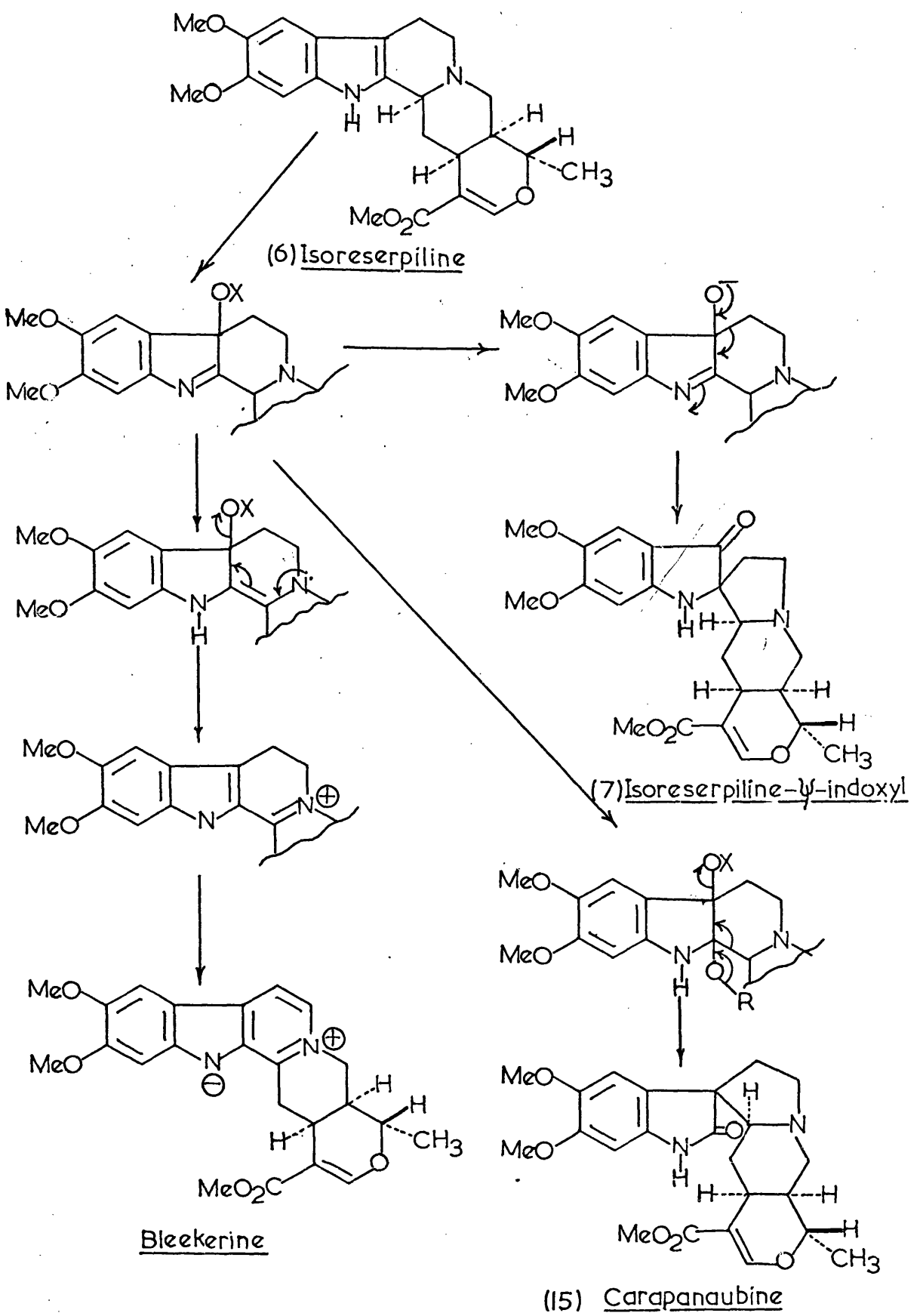


Corynanthe system

Since we have shown that lead tetra-acetate oxidation of isoreserpiline, a "Corynanthe type" alkaloid, yields the alkaloid bleekerine (12, $R_1 = R_2 = OMe$) there appears to be a direct correlation between *in vitro* and *in vivo* reactions leading from isoreserpiline (6) to bleekerine carapanaubine (15) and isoreserpiline-ψ-indoxyl (7) (Scheme 4).

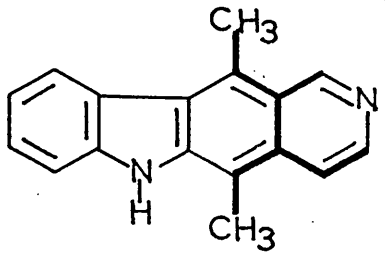
The Aspidosperma and Iboga systems arise via the Strychnos type by an initial $\alpha \rightarrow \beta$ rearrangement of the indole moiety of geissoschizine (33)[56,57].

Scheme 4

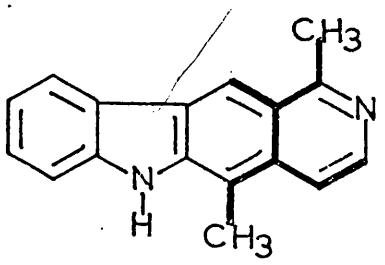

MeO
MeO
N
H
H
H
H
H
CH3
MeO2C
O
(6) Isoreserpiline
OX
O⁻
N⁺
(7) Isoreserpiline-ψ-indoxyl
Bleekerine
R
(15) Carapanaubine

The ellipticine 'Alkaloids'*

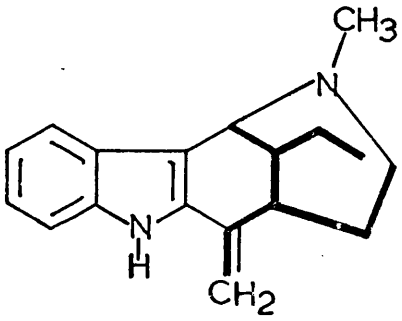
This unique group of indole alkaloids when first recognized presented somewhat of a shock to those adept in the art of biogenetic speculation because the number of carbon atoms between the indole-β-position and the basic nitrogen was not two. Prior to their discovery a tryptamine residue could be discerned in all of the known indole alkaloids. In common with other indole alkaloids with which they co-occur, however, the 'ellipticine group' contains the C_9-Corynanthe-Strychnos C_9 unit (outlined in heavy type in the structural formulae 2, 34 and 35).



(2)



(34)

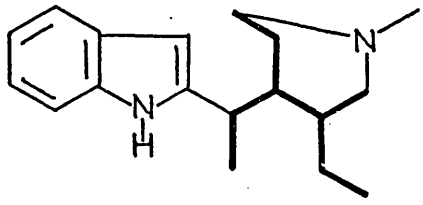


(35)

* Ellipticine (2) olivacine (34) and uleine (35)

The fundamental problem was originally defined by Schlittler and Taylor[58] when they asked "are these substances being derived from a C_9-element, ammonia and indole, or from tryptamine followed by loss of two carbon atoms"?

Woodward[39] had suggested the intermediate (36) to relate uleine and ellipticine, but did not discuss the mode of formation of this precursor.

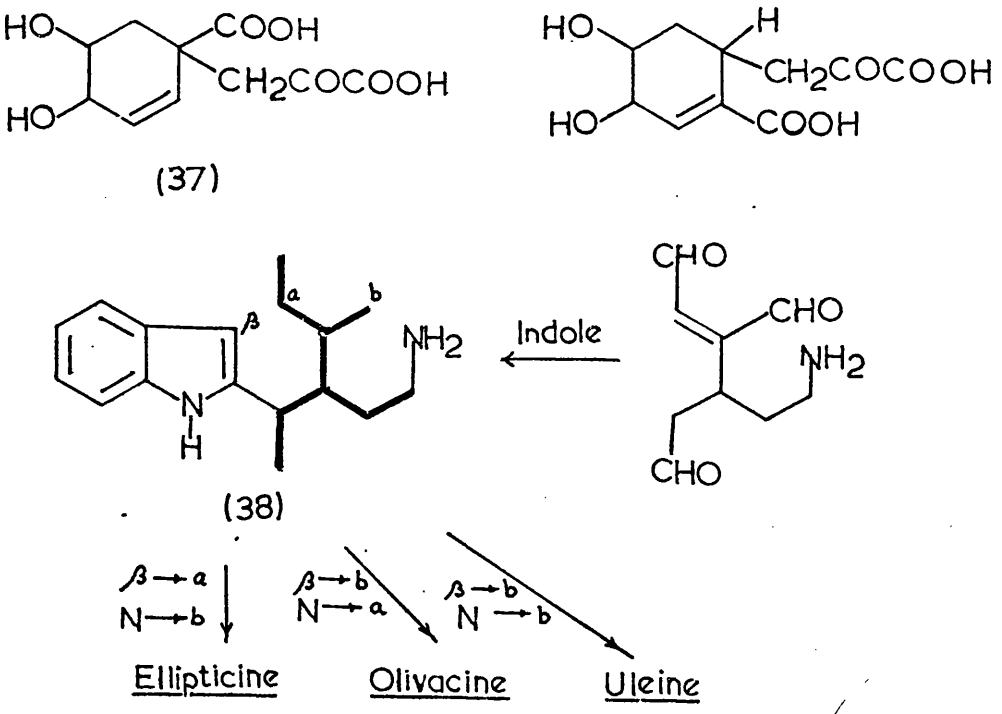


(36)

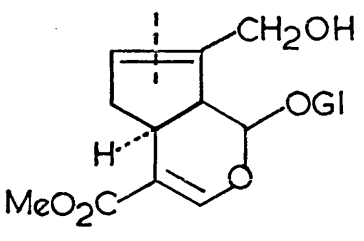
An ingenious speculation (Scheme 5) was submitted by Boit[59] who suggested hydrated prephenic acid (37) as the pregenitor of the C_9-unit: the intermediate (38) which is essentially the same as Woodward's structure (36) could then be formed by reaction with ammonia and indole. Ring closure to ellipticine (2), uleine (35) and olivacine (34) then follows.

In the light of present evidence concerning the biogenesis of the terpenoid indole alkaloids this proposal is almost certainly incorrect since a terpenoid precursor seems much more likely.

Scheme 5

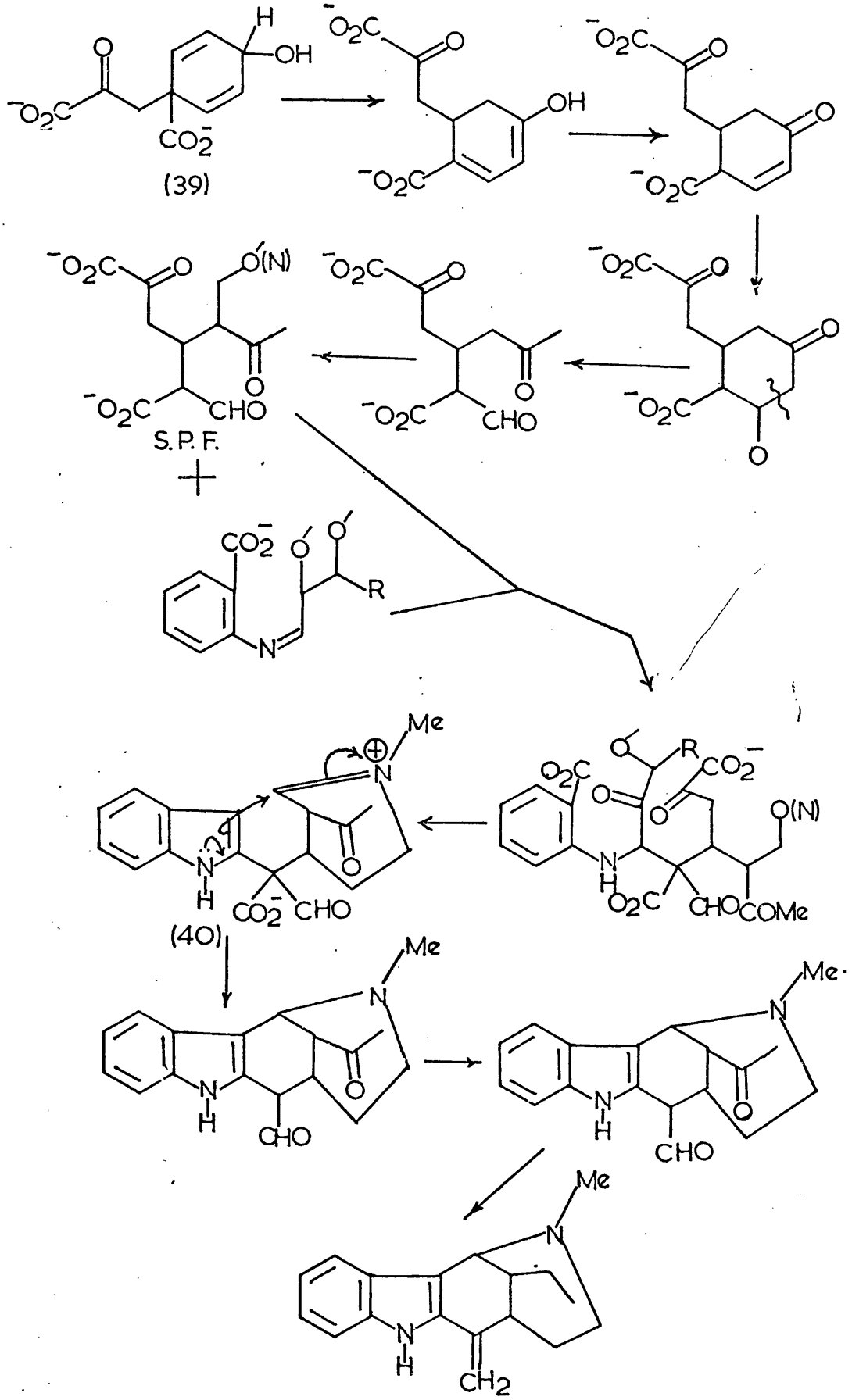


Wenkert[60] on the other hand rejects indole as an intermediate and suggests that tryptamine or rather tryptamine precursor is involved. This author suggests two alternative routes to the non-tryptamine portion. One, from prephenic acid (39), is incorporated in Scheme 6, outlining the generation of uleine; the formation of ellipticine and olivacine from the intermediate (40) is readily visualized. However, Wenkert also recognized that the cyclopentane monoterpene genepin (41) could, by oxidative cleavage, furnish an analogous system to seco-prephenate formaldehyde (S.P.F., Scheme 6).



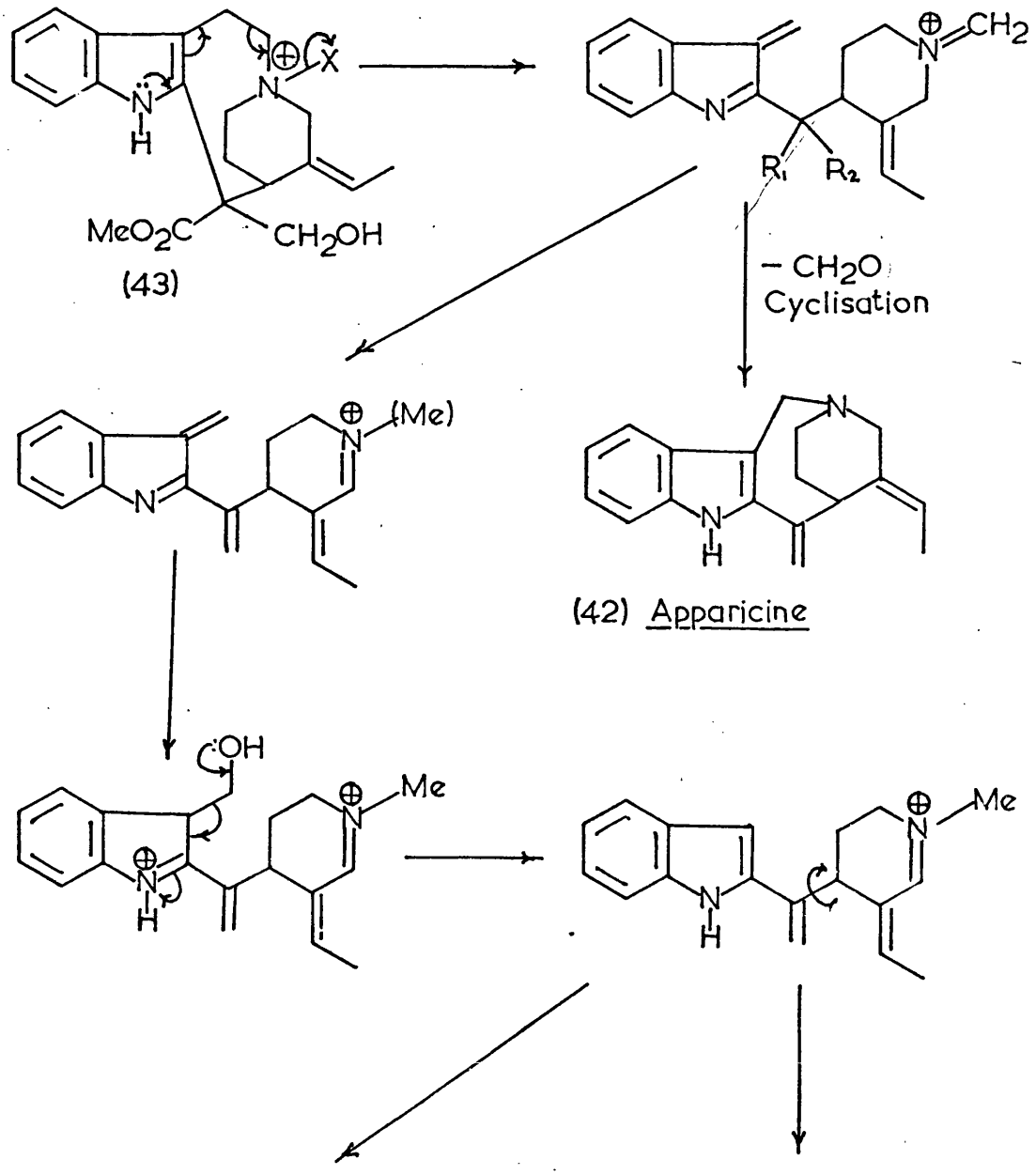
(41)

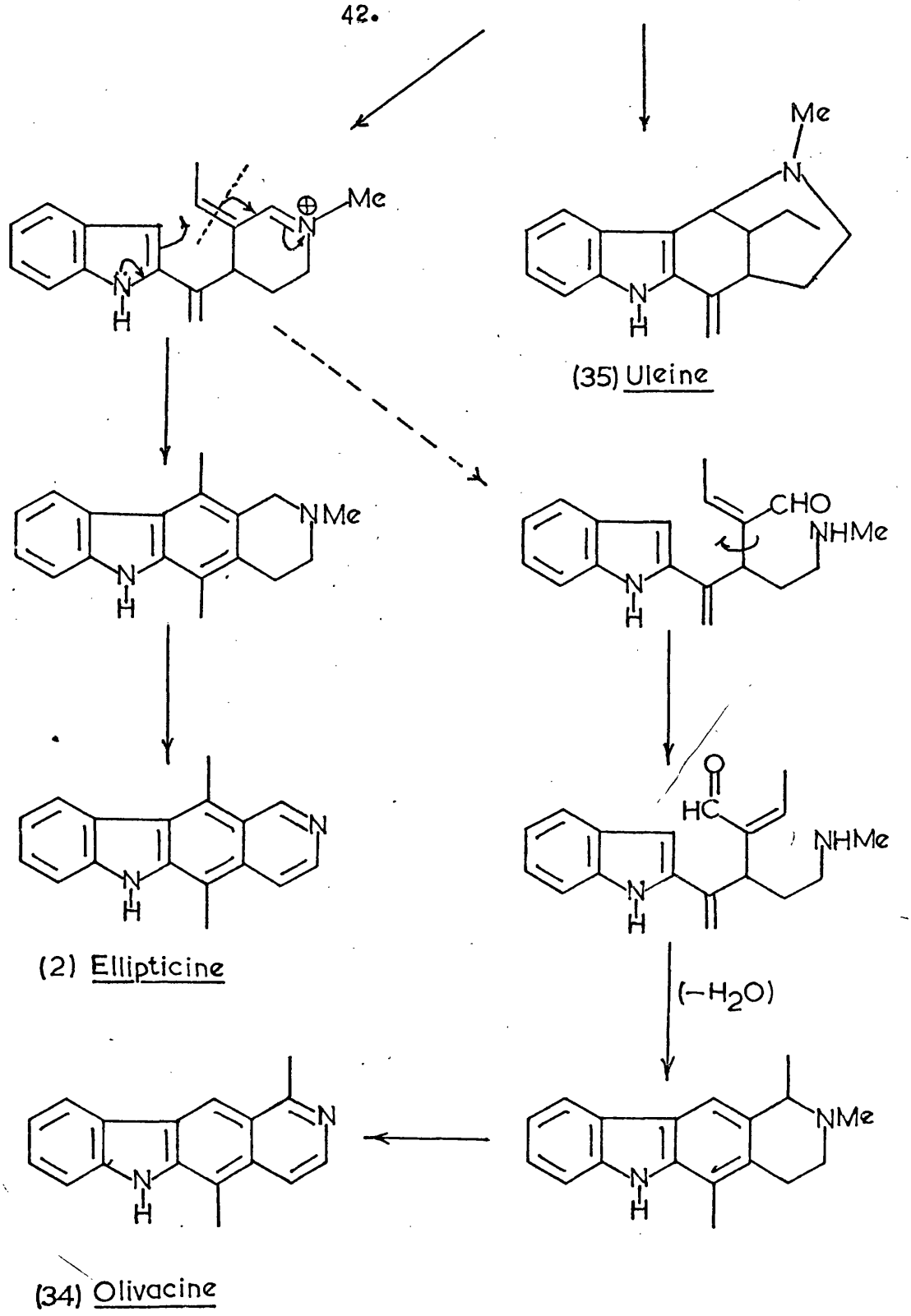
Scheme 6



Recently a postulation[61] which directly relates the ellipticine alkaloids with the terpenoid indole alkaloids has been published (see Scheme 7). However, while the formation of apparicine (42) is supported by preliminary feeding experiments, incorporation of stemmadenine (43) into uleine was not observed[62].

Scheme 7

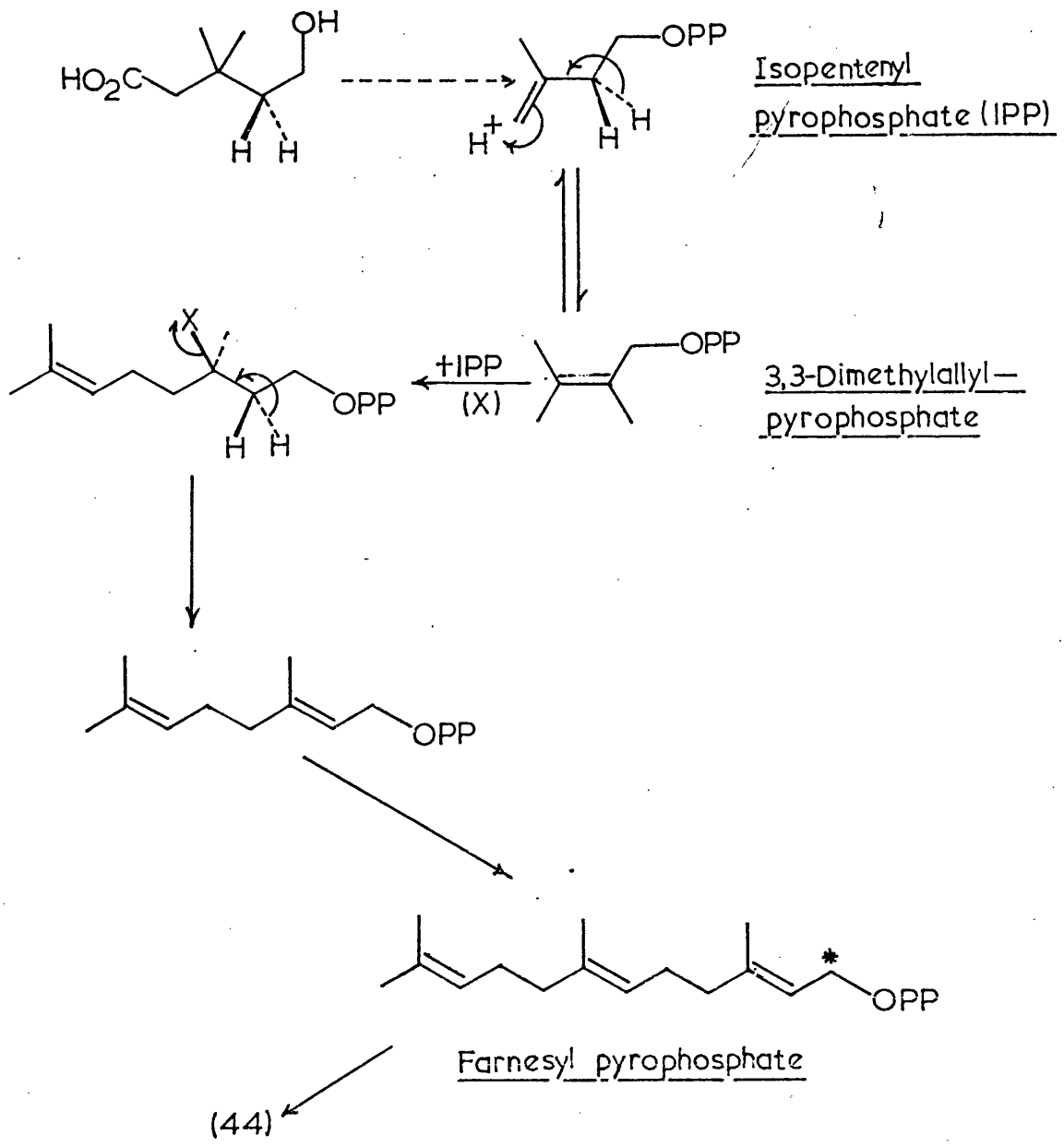


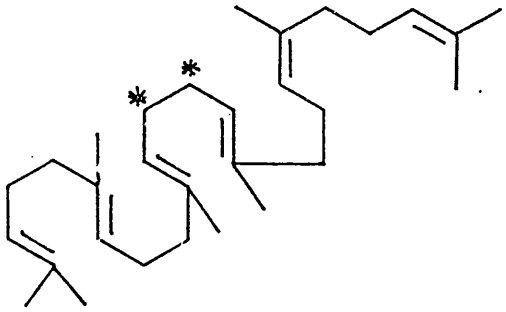

Me
N
Me
N
H
(35) Uleine
NMe
CHO
NHMe
N
H
(2) Ellipticine
HC
O
NHMe
(−H2O)
NMe
(34) Olivacine

The Phytosterols

The biosynthesis of sterols in plants follows a pathway close to the formation of cholesterol in animal tissue[63]. Thus squalene (44) is derived from mevalonic acid (45)[64] by the same stereospecific enzymatic processes[65] (see Scheme 8). Although the exact mechanisms involved are still in doubt it has been shown that chain extention occurs with inversion of configuration and therefore is akin to a classical S_N2 mechanism.

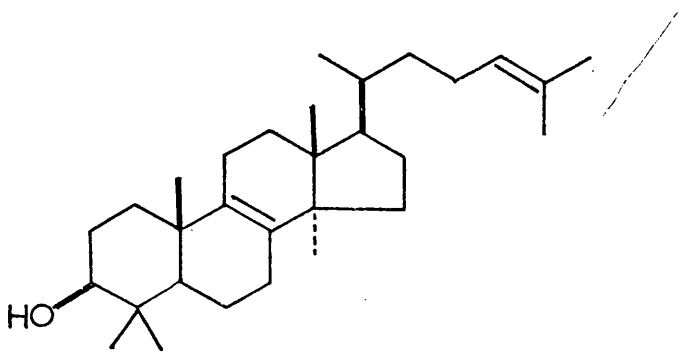
Scheme 8.





(44)

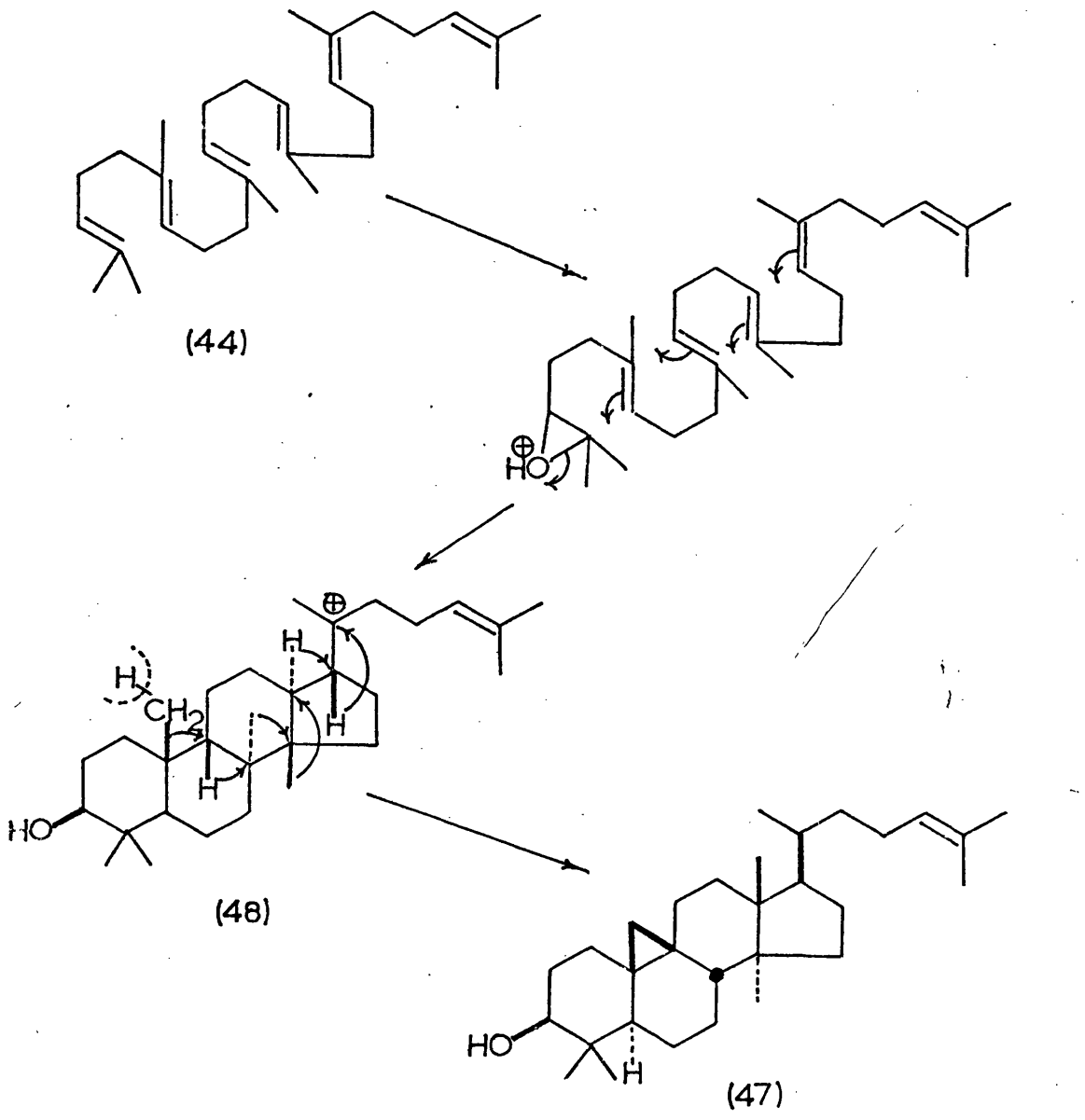
Whereas lanosterol (46) is an intermediate in the formation of cholesterol by mammals, this substance is not generally found in plants and evidence has accumulated that it is not on the direct route to phytosterols[66].



(46)

Tracer studies[67] suggest that cycloartenol (47), which can be hypothetically formed from the 'chain-boat-chain-boat--unfolded' configuration of squalene is the intermediate (Scheme 9).

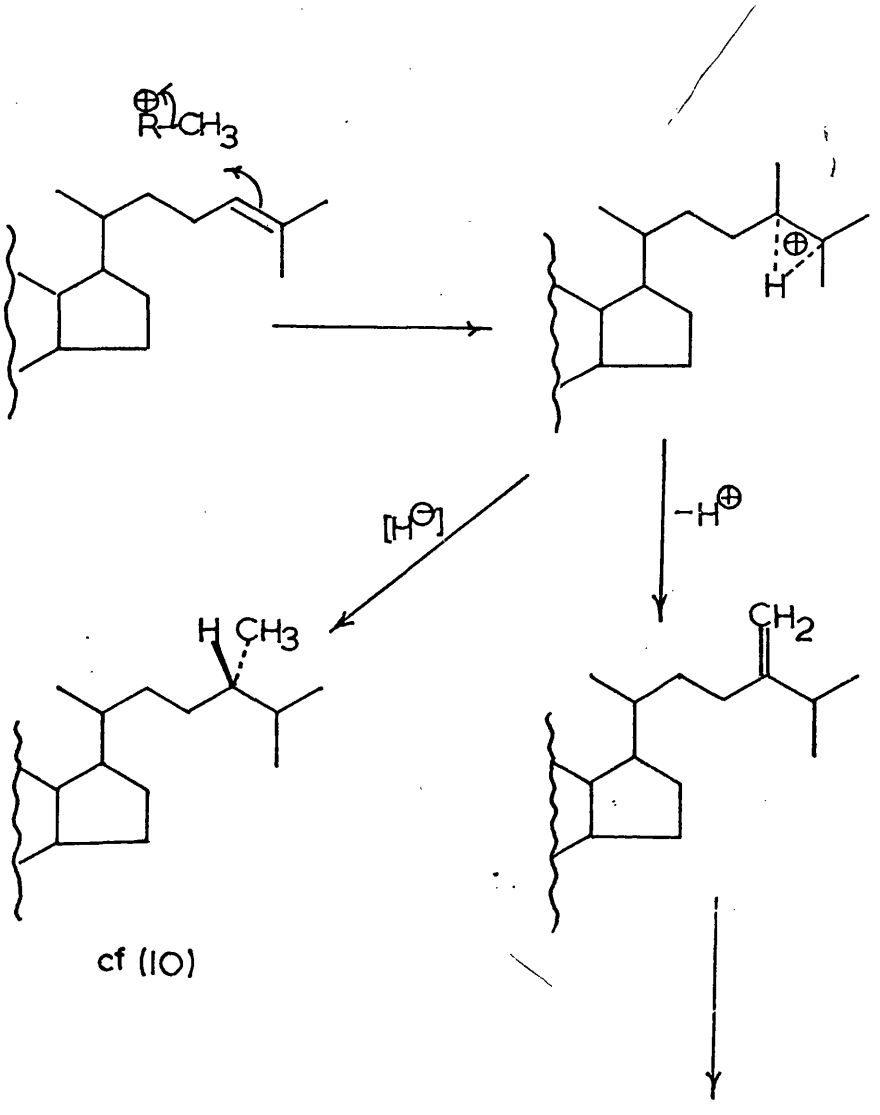
Scheme 9

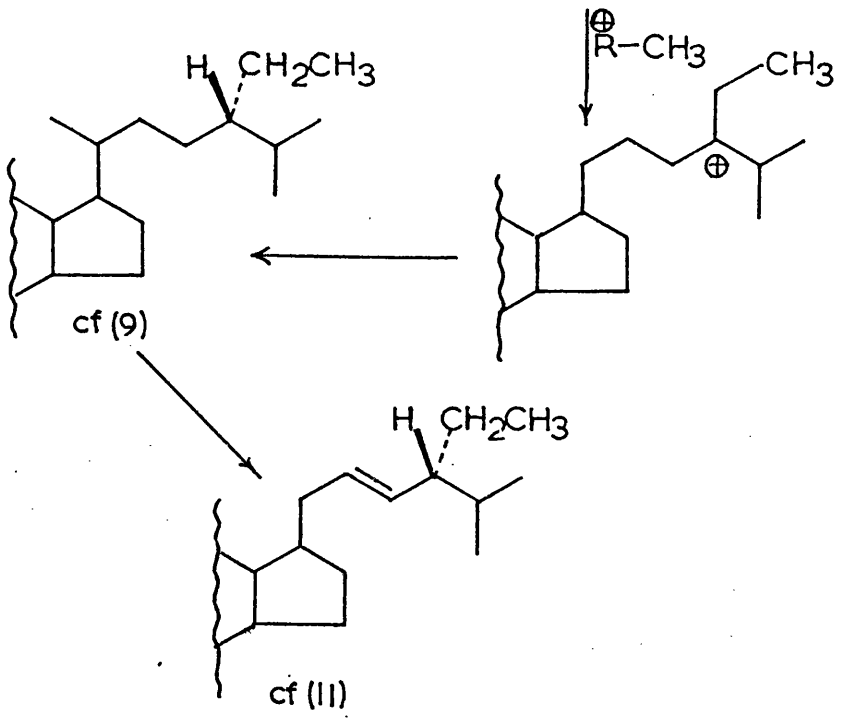


A noteworthy feature of this route, which has been substantiated by feeding experiments[68], is the formation of the cyclopropane ring with hydrogen migration in the intermediate (48). Since this mechanism appears to contradict the stereoelectronic requirements in that both functions involved are on the same side, an intermediate enzymatic step is probably involved.

The exact sequence for the generation of phytosterols from cycloartenol (47) is obscure. Loss of methyl groups may or may not occur before side chain alkylation[69,70], although it is believed[63] that the C_{14}-Me group is removed before the gem-dimethyl units in the biosynthesis of mammalian cholesterol. Side chain alkylation, achieved by methyl transfer from S-adenosylmethionine[71], gives rise to a methyl group at C_{24} as in campesterol (10) or, by further alkylation[72] to give an ethyl derivative as in β-sitosterol (9). Stigmasterol (11) apparently arises from β-sitosterol by desaturation[70] (See Scheme 10).

Scheme 10





Experimental to Part 1

Extraction of the Bark

The finely divided bark (500g) was exhaustively extracted with ethanol (7l). Concentration of the dark red ethanolic solution under reduced pressure gave a thick brown gum (2g). Four major components were discernable on chromatographic analysis (Al_2O_3, $CHCl_3$) three of which were recognized as isoreserpiline (6), ellipticine (2) and 9-methoxyellipticine (1) (Rf 0.85, 0.6 and 0.46 respectively) by comparison with authentic samples.

The crude extract was absorbed on Al_2O_3 (8g) and transferred to an alumina column (150g). Eluting with mixtures of $CHCl_3$ - petrol (60-80), chloroform and finally methanol in chloroform, three major portions were collected:- A (50% $CHCl_3$ in petrol), B (75 - 100% $CHCl_3$ in petrol) and C (5 - 100% MeOH in $CHCl_3$).

Fraction A

A greenish sticky solid was obtained when the solvent was evaporated and TLC analysis showed it contained three principal components. Further chromatography (30g Al_2O_3, eluting with 50% $CHCl_3$ in petrol 60-80°) affected partial separation. Fractions 1 - 14 (total 280ml) gave an off white solid residue (71mg) and on trituration with methanol a mixture (36mg) of β-sitosterol (9), stigmasterol (11) and campesterol (10) crystallized together.

Chromatographic analysis indicated that further partitioning of the remaining fractions was not justified and when combined and evaporated these gave a small quantity of isoreserpiline contaminated with isoreserpiline-γ-indoxyl. These components were identified by mass spectral analysis and TLC comparison with authentic specimens.

Fraction B

On evaporation of the solvent from this fraction, a dark brown semi-crystalline product formed which was effectively purified by trituration with methanol. When filtered and crystallized from ethanol (prisms) the new alkaloid bleekerine (12, $R_1 = R_2 = OMe$) (45mg) was obtained m.p. 276-7°, $[\alpha]^{22.5}_{546nm}$ + 612 (MeOH), Rf Al_2O_3(EtOH) 0.46 (bright blue fluorescence under UV light). Precision M.S. 408.1690, $C_{23}H_{24}N_2O_5$ req. 408.1685. (Found: C, 67.80; H, 6.0; N, 7.10. $C_{23}H_{24}N_2O_5$ req. C, 67.60; H, 5.9; N, 6.90%).

The filtrate was evaporated to dryness giving a brown gum. Solidification was brought about by repeated trituration with ether, finally giving an off-yellow solid. Treatment of an aqueous solution of this material with perchloric acid (5 drops) furnished holeinine

perchlorate (13, X = ClO_4) (7mg) which crystallised from water m.p. 224-6° (lit.[22], 228 - 231°). Comparison by I.R. and m.m.p. with a sample prepared from an authentic specimen of holeinine kindly supplied by Professor P.J. Scheuer, showed that the two specimens were identical.

The combined ether layers were evaporated to give a crude mixture of isoreserpiline and sterols (57mg). This material combined with crude isoreserpiline obtained from fraction A was applied to a preparative layer (0.8mm) Al_2O_3 plate and eluted repeatedly with 50/50 $CHCl_3$-petrol (60-80°). Isoreserpiline (50mg) and sterol mixture (10mg) were extracted ($CHCl_3$) from the bands at R_f's 0.65 - 0.75 and 0.90 - 1.00 respectively.

Fraction C

TLC (Al_2O_3, $CHCl_3$) analysis of this fraction indicated the presence of several components, the most prominent having R_f values at: 0.90 (visible only after development with iodine), 0.58 (blue fluorescence under U.V.) and 0.46 (red fluorescence under UV).

On partial evaporation a yellow solid was deposited, thereafter the mixture was allowed to stand overnight before filtering off relatively pure 9-methoxyellipticine (1). Recrystallization from ethyl acetate afforded fine yellow needles, m.p. 280-2: (lit[1] 280-5°). R_f 0.72 (Al_2O_3, 5% MeOH in chloroform, red fluorescence in UV light); a further crop of 9-methoxyellipticine crystallized (total 791mg). Finally complete evaporation of the filtrate gave a slight deposit shown by mass spectroscopy and TLC (Al_2O_3, $CHCl_3$, R_f's 0.60 and 0.46, blue and red fluorescence under UV light respectively) to be a

mixture of ellipticine and 9-methoxyellipticine. The residue was extracted with hot benzene filtered and partially evaporated to cause crystallization of ellipticine (13mg), although still contaminated with some 9-methoxyellipticine.

The remaining portion of fraction C was rechromatographed over Al_2O_3 (30g). Development was commenced with 50/50 $CHCl_3$ in pet·ol changing to chloroform and then 5, 10, 20 and 100% MeOH in $CHCl_3$ mixtures. Monitoring by TLC, four crude fractions were collected: D, E, F and G.

Fraction D

Removal of the solvent in vacuo rendered an almost colourless solid (9mg). Comparison by TLC and mass spectroscopy revealed it was the sterol mixture previously isolated.

Fractions E and G

These fractions appeared by TLC (Al_2O_3, $CHCl_3$) to have similar constitution. Therefore both were combined (total 107mg) and triturated repeatedly with ethyl acetate, eventually yielding almost pure 9-methoxyellipticine (33mg). (overall yield 824mg, 0.165%).

Fraction F

An amorphous tan coloured solid (300mg) remained on removal of the solvent. Preliminary solubility investigation established it was in the main, very soluble in alcohol and water. Crystallization attempts with ethyl acetate, $CHCl_3$, acetone, methanol and water mixtures failed. A suspicion that this material contained quaternary salts was given substance when a silver nitrate test was positive.

However, washings with hot petrol (60-80°) followed by hot dichloroethane were successful in effecting considerable purification and this treatment eventually provided a crisp yellow solid (127mg).

An aqueous solution of this product was prepared and filtered. On evaporation and crystallization of the residue from $CHCl_3$ holeinine (Cl /Br mixture) was obtained as a colourless solid (75mg) (giving a total yield of 80mg, 0.016%).

Extraction of the Wood.

The dried pulverized wood (500g) was subjected to continuous extraction with ethanol (5 l) by the Soxhlet technique. After filtering off the woody material and evaporating in vacuo a dark brown resinous product remained (0.113g).

A 20cm. preparative layer (0.8mm thick) Al_2O_3 plate was prepared and a solution of the wood extract in $CHCl_3$ applied evenly to one edge. On repeated elution with 1% MeOH in $CHCl_3$ a red fluorescent band formed. Recovery of the organic matter was achieved by continuous extraction with chloroform yielding crude 9-methoxyellipticine which crystallized from ethyl acetate as yellow needles (30mg, 0.006%) m.p. 276-80 (lit[1] 280-285). R_f 0.7 (Al_2O_3, 6% MeOH in $CHCl_3$).

Similarly, continuous extraction ($CHCl_3$) of the band at the solvent front (detected by treating a pilot plate with 50% H_2SO_4 and baking) gave a white solid (5mg). Mass spec: m/e 414.3844 ($C_{29}H_{50}O$), 412.3705 ($C_{29}H_{48}O$) and 400.3705 ($C_{28}H_{48}O$) corresponding to β-sitosterol, stigmasterol and campesterol respectively. G.L.C.: 2.5% OVI on chromasorb W, AW-DMCS 200°, R_f's (with respect to β-sitosterol) 1.0, 0.87, 0.74. These values are closely similar to those reported

by Rowe[73] and assignments were confirmed by reference to authentic samples.

The remaining adsorbant was scraped from the plate and on continuous extraction ($CHCl_3$) gave a brown gum (21mg). Comparison by TLC (Al_2O_3, $CHCl_3$) and mass spectra showed the compounds: ellipticine, isoreserpiline and isoreserpiline-ψ-indoxyl to be present.

Reduction of Bleekerine with $NaBH_4$

Bleekerine (10mg) in MeOH was treated with $NaBH_4$ (20mg) and the reaction mixture was heated under reflux until no further change was observed in the UV spectrum. Removal of the solvent gave a solid residue which was treated with H_2O (5ml) and extracted with $CHCl_3$. Evaporation of the dry solvent phase yielded a gum which was purified by preparative TLC (Al_2O_3) eluting with 50% $CHCl_3$-petrol. Removal of a band R_f 0.6-0.8, followed by extraction with $CHCl_3$ and repeated chromatography afforded isoreserpiline (1.8mg), identical with an authentic specimen (TLC, IR, MS).

Oxidation of Isoreserpiline with $Pb(OAc)_4$

A solution of isoreserpiline (20mg) in HOAc (2 ml) maintained at 60° was treated dropwise with $Pb(OAc)_4$ (30mg) in HOAc (10ml). The addition was stopped when the UV spectrum of the product no longer showed the presence of the indole chromophore. Removal of HOAc under reduced pressure gave a dark red oil, this was dissolved in CH_2Cl_2 and shaken with 2 N Na_2CO_3 solution. The dried solvent layer was reduced in volume and applied to preparative TLC plates coated with Al_2O_3. The plates were developed with $CHCl_3$-MeOH (1:1) and viewed in UV light.

A band R_f 0.6–0.85 was removed from the plates and worked up to yield a mixture of isoreserpiline and isoreserpiline- -indoxyl, whereas a band at R_f 0.5–0.35 showing bright blue flourescence was extracted to give a semi-solid product. This was rechromatographed, affording 0.8mg of pure material identical (MS, GLC and m.m.p.) with bleekerine from Bleekeria vitiensis.

PART II

PART II

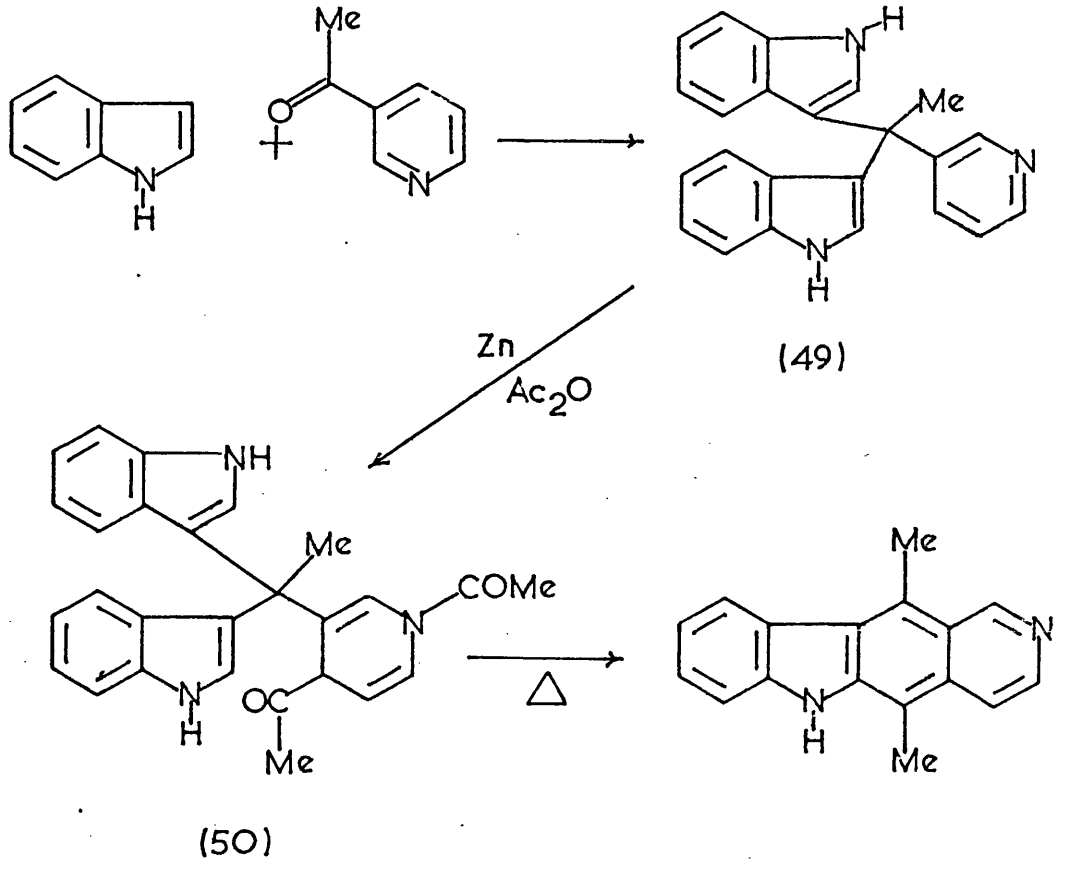
INTRODUCTION

In the second part of this thesis synthetic work carried out by the author to implement some of the Hantsch proposals[15] is discussed. Prior to this it is appropriate to review present methods available for the preparation of ellipticine derivatives

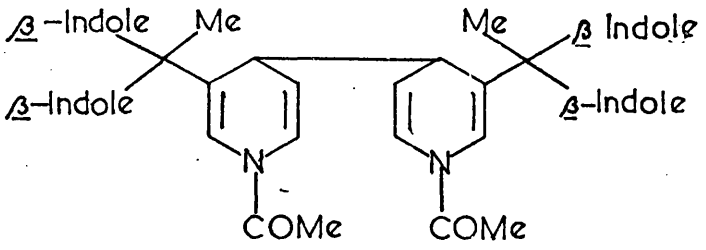
SYNTHETIC ROUTES TO ELLIPTICINE DERIVATIVES

A remarkable synthesis (Route 1) of ellipticine[39] followed closely upon the discovery of this alkaloid and served to confirm the original structural assignment. In this work the intermediate (49) was formed by reaction of indole with 3-acetylpyridine; reductive acylation of this material with zinc and acetic anhydride then afforded the compound (50). Cyclisation of this to ellipticine was achieved by pyrolysis and sublimation. The yield in the final reaction was 2%.

Route 1.



Interestingly, the reductive acetylation of pyridines requires the prior formation of a dimer, in this case (51), which then disproportionates into starting material and a 1,4-diacetyl-1,4-dihydropyridine; the latter usually undergoing oxidation to the corresponding 4-acetylpyridine.



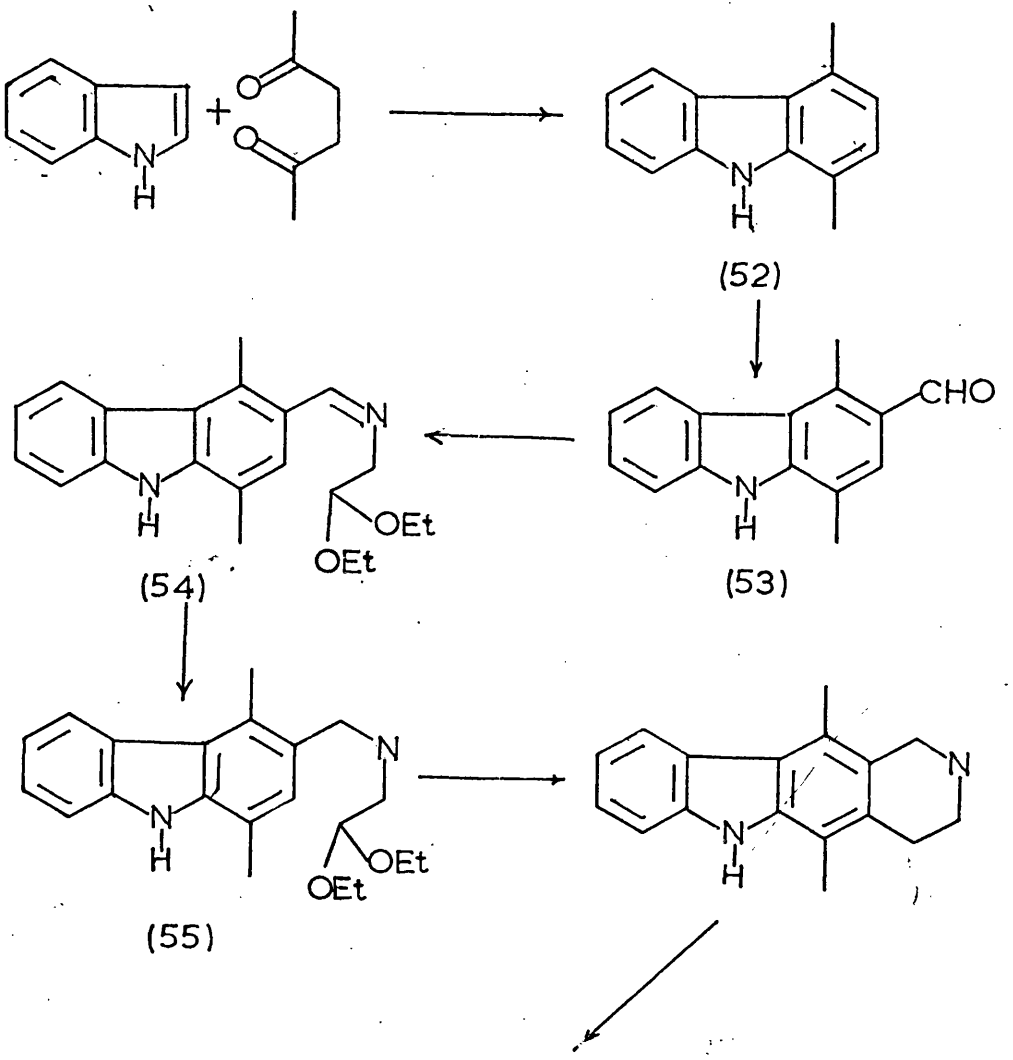
(51)

Normally, because of the participation of the dimer, this reaction is very sensitive to steric factors and frequently fails with substituted pyridines (see page 70). The formation of the highly hindered dimer (51) is thus very unusual and significantly the yields of the intermediates in the synthesis were not quoted. Clearly, this approach has no practical application.

A more conventional synthesis (Route 2) was adopted by Cranwell and Saxton[11]: condensation of indole with hexan-2,5-dione gave an optimized 36% yield of 1,4-dimethylcarbazole (52) which was then converted into the 3-formyl derivative (53), contaminated with some of the 3,6-diformyl-product.

Although the Schiff's base (54) was prepared from (53) in good yield, attempts to ring close this compound failed and it was necessary to proceed <u>via</u> the dihydro-derivative (55). This requirement diminished the overall yield of ellipticine to 7%

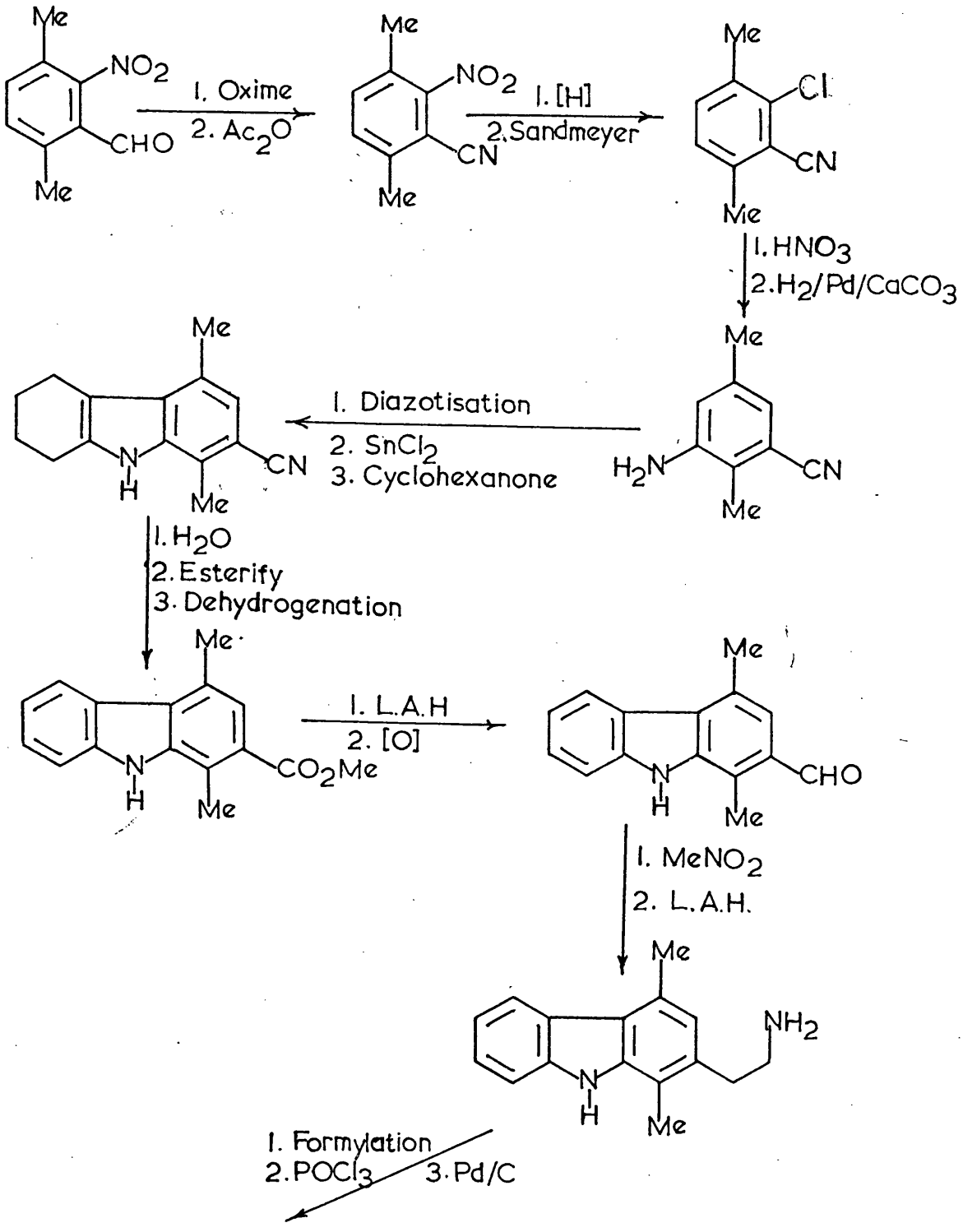
Route 2



ELLIPTICINE

Following on from the development two other syntheses were reported, neither has practical utility. In the first[74] the tetra-cyclic system of ellipticine was constructed by the elaborate scheme summarized in route (3).

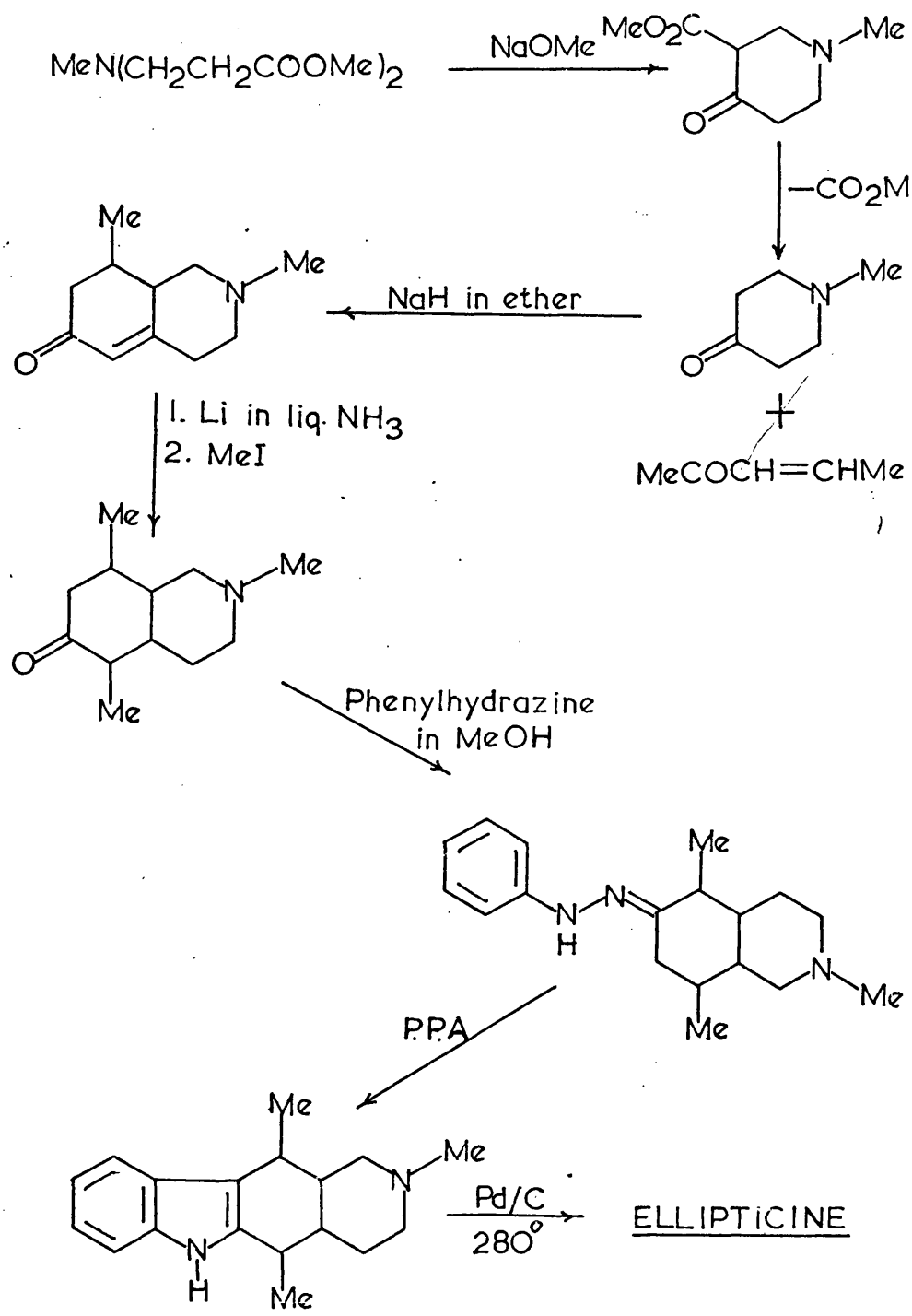
Route 3



ELLIPTICINE

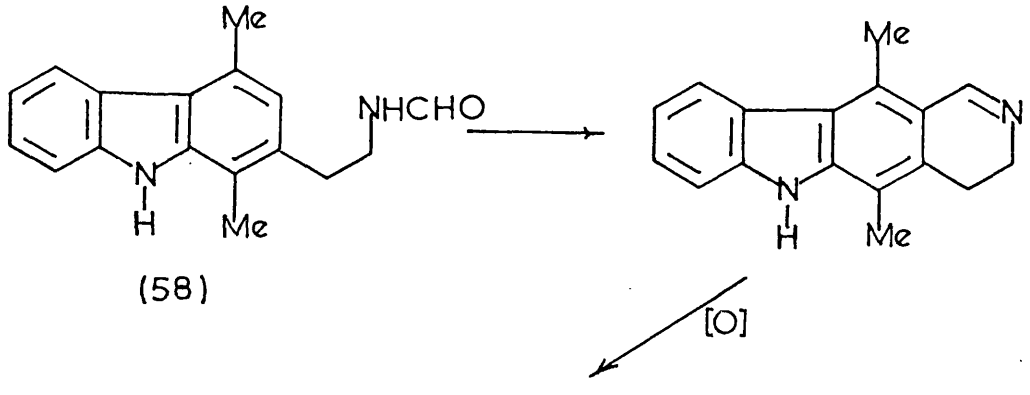
The second[75] adopted the unusual approach of constructing the alkaloid right to left (see route 4). Unfortunately the promise of model reactions was unfulfilled in the actual synthesis and yields were poor.

Route 4



At this point Dalton and his co-workers[10] realized the potential of Cranwell and Saxton's original synthesis by using 100% phosphoric acid to cyclise the intermediate (54) directly to ellipticine, thus obviating the two final steps of the original scheme.

During this study it was found that electron donating groups in C-6 position of derivatives of (54) assisted ring-closure, whereas electron withdrawing functions had the opposite effect. However, electron donating groups in the non-methylated benzenoid ring of carbazoles (type 52) presented another problem since on formylation these gave, in addition to the required 6-formyl derivative (type 53), carbazoles containing the formyl group in the same ring as the electron donating substituent. Nevertheless accepting these limitations a number of 9-substituted ellipticines were prepared for biological testing. A variation of this synthesis has been patented by a Swiss group[76], here cyclisation of the 2-substituted carbazole (58) with phosphorus oxychloride furnished 3,4-dihydroellipticine which was then dehydrogenated to ellipticine.



(58)

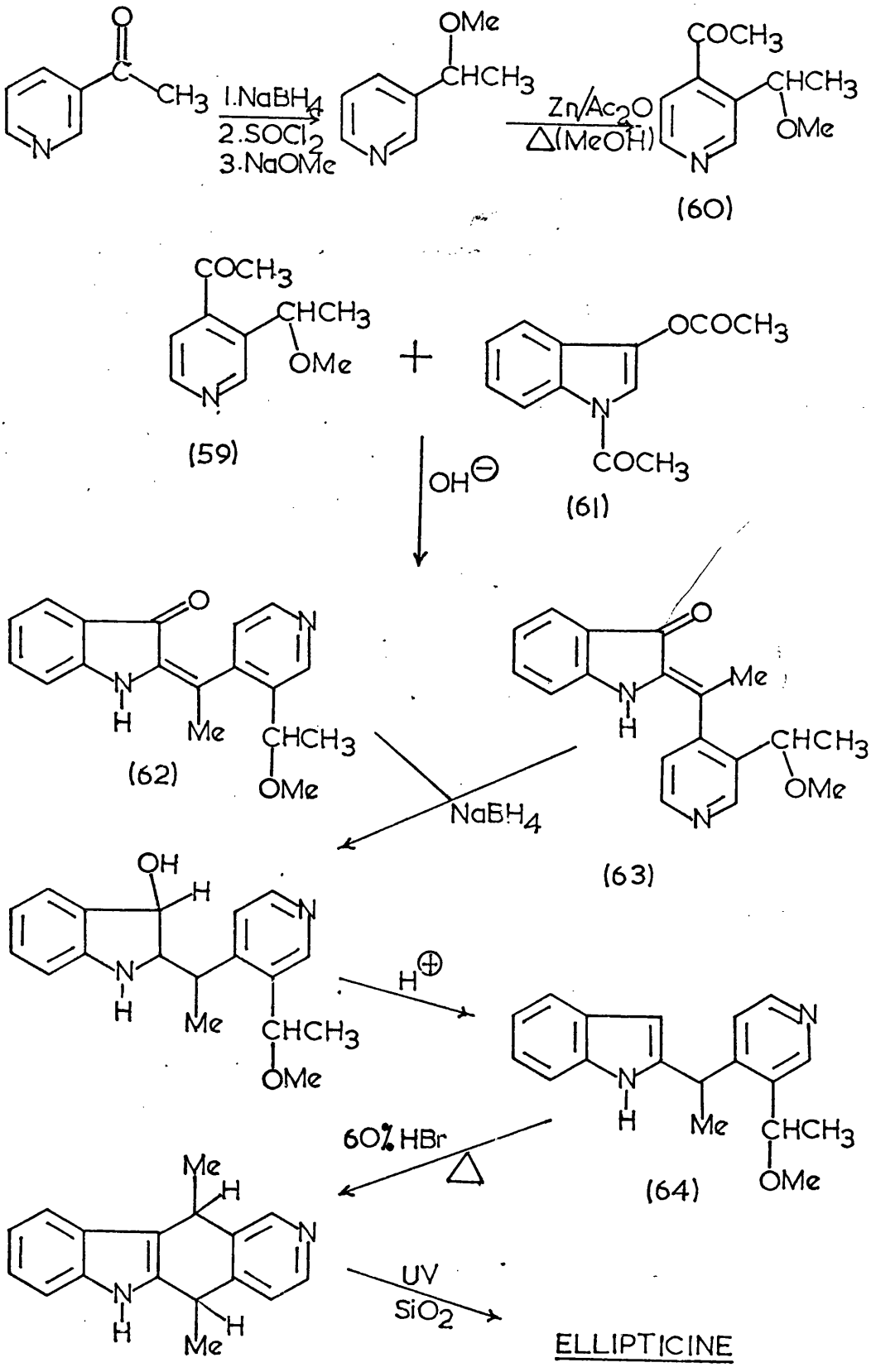
ELLIPTICINE

Potentially the most rewarding and versatile synthesis[77] of ellipticine resulted from previous work in this laboratory. Here the 4-acetylpyridine (59), prepared from 3-(1-methoxyethyl)-pyridine (60) by reductive acetylation and subsequent aromatization was condensed with 1,3-diacetylindoxyl (61) to give a mixture of the geometric isomers (62) and (63) in 75% yield. Reduction and dehydration of the isomers then gave the indole (64), again in excellent yield, and this when heated with aqueous hydrobromic acid afforded ellipticine. See Route 5.

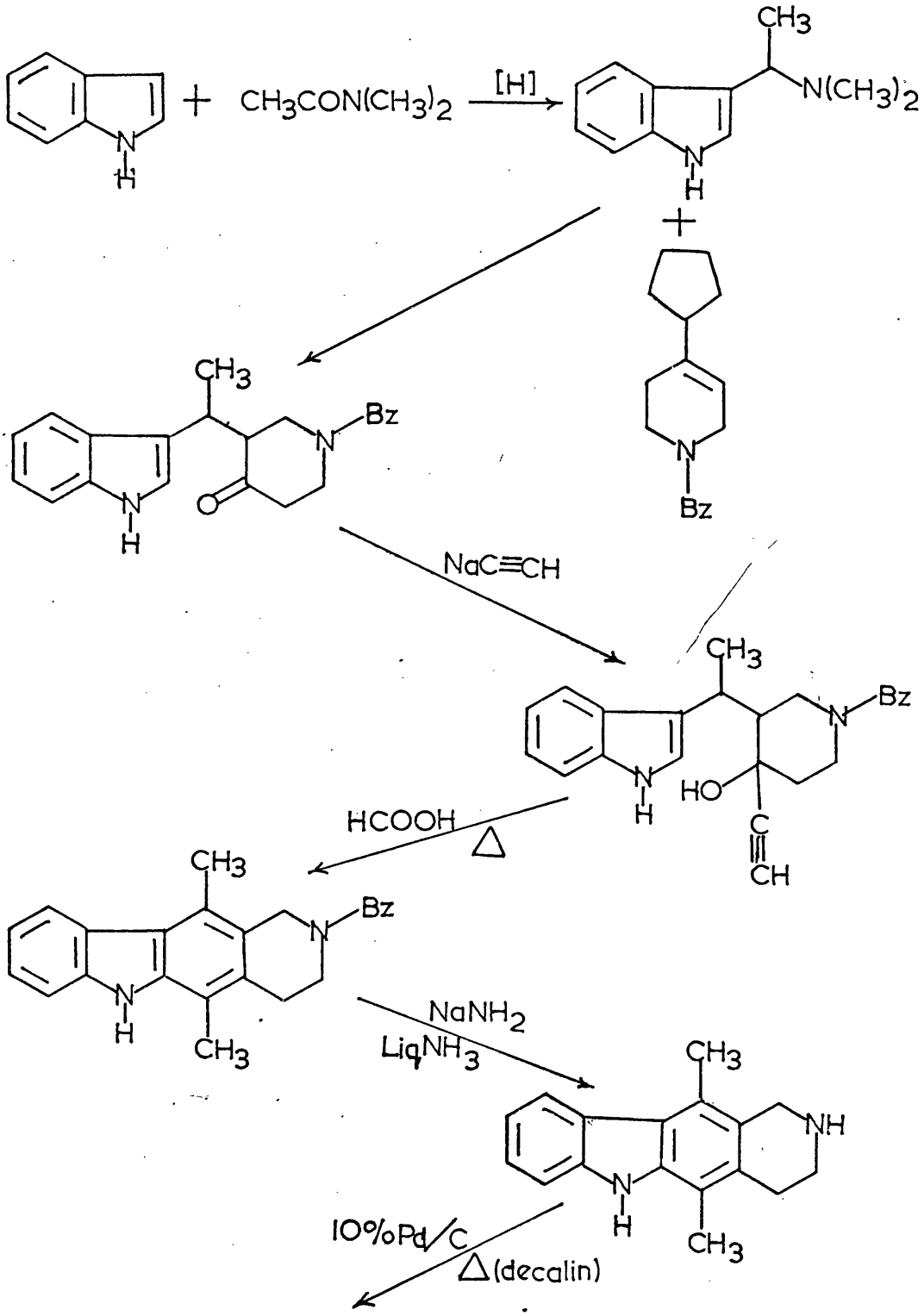
Apart from a much improved yield (31% overall) this route suffered none of the limitations of Dalton's synthesis and consequently has been the basis for much of the work described in this section of the thesis.

More recently Le Goffic and his colleagues[78] have pioneered yet another ellipticine preparation, in this six stage process (Route 6) ellipticine was constructed from indole by an initial Baeyer Villiger reaction. On the face of it numerous ellipticine should be easily formed by this synthesis, but in our experience many substituted ellipticines are thermally unstable and the necessity of the ultimate dehydrogenation step in Goffic's approach is undesirable. (cf Mosher et al[79]).

Route 5


1.NaBH4
2.SOCl2
3.NaOMe
Zn/Ac2O
Δ(MeOH)
(60)
(59)
OH⊖
(61)
(62)
(63)
NaBH4
H⊕
(64)
60% HBr
Δ
UV
SiO2
ELLIPTICINE

Route 6



ELLIPTICINE

Merely by trial and error, an attempt to improve the biological activity of a molecule such as ellipticine in which there are several sites where substitution may occur requires the preparation of a very large number of derivatives. Hantsch[15], however, has indicated how a careful choice of substituents may achieve a more direct approach to this goal.

Thus by considering three main parameters: hydrophobic, electronic and steric changes a judicious choice of those substituents most likely to indicate trends in structure-activity relationships can be made.

An assessment of the way in which the molecule is distributed in the biological system is achieved by considering the partition coefficient (P) of the compound between octanol and water. This non-specific hydrophobic factor, as Hantsch calls it, is an additive property of the functional units from which the complex molecule is derived and thus for 5,11-desmethylellipticine log P is calculated as follows:

πC_6H_5NH log P(isoquinoline) = 1.37 + 2.08 = 3.45

where πC_6H_5NH log $P(C_6H_5)_2NH$ - log PC_6H_5

This approximation correlates well with the experimentally derived value of 3.56.

In general most useful drugs have a value of log P in the range 0-6, this factor alone limits the use in derivatives of many potential substituents and in the case of ellipticines some of the most useful functions are listed in table IV.

Table IV

Function	π	σ_{para}	σ_{meta}
$-CO_2H$	-4.36	0.00	-0.10
$-SO_2NH_2$	-1.82	0.57	0.46
$-SO_2CH_3$	-1.63	0.72	0.60
$-CONH_2$	-1.49	0.36	0.28
$-NHCONH_2$	-1.30	-0.24	-0.03
$-NH_2$	-1.23	-0.66	-
$-NH_2COCH_3$	-0.97	0.00	0.21
$-OH$	-0.67	-0.37	0.12
$-CN$	-0.57	0.66	0.56
$-OCH_3$	-0.02	-0.27	0.12
$-H$	-0.00	0.00	0.00
$-F$	0.14	0.06	0.34
$-CH_3$	0.50	-0.17	-0.07
$-Br$	0.86	0.23	0.39
$-C_2H_5$	1.00	-0.15	-0.07
$-SCF_3$	1.44	0.50	0.40
$-C_6H_5$	1.96	-0.01	0.06
$-OC_6H_5$	2.08	-0.32	0.25

The values $\sigma_{(para)}$ and $\sigma_{(meta)}$ are the Hammett constants used to assess the electronic effects

$$\sigma = \log \left(\frac{K_x}{K_H}\right)$$

Where K_X is the dissociation constant of a substituted benzoic acid and K_H the dissociation constant of benzoic acid itself.

Two types of situations arise when attempting to modify a drug. First there is the case where the preparation of a set of derivatives requires less time than the evaluation of their activity. In this case a number of derivatives are made where the substituents are chosen so that changes in hydrophobic and electronic effects are well delineated. Since the synthetic problem is not great this course of action is repeated until the requirements for activity are clearly revealed.

On the other hand when the time spent for preparation is longer than the testing time derivatives should be chosen such that the maximum amount of information is gathered from each derivative. Ideally substituents of widely differing properties are selected first to give the range, then in a limited number of moves the activity is optimised. A detailed discussion of this approach is given by Hantsch[15].

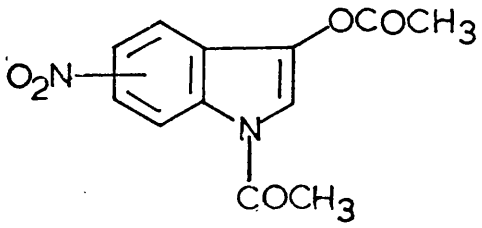
Initially we considered the former to be more appropriate to the ellipticine case and we therefore directed our efforts towards preparing 9-nitroellipticine which could, by straight forward processes, be converted to several other derivatives. Unfortunately the synthesis of 9-nitro-ellipticine could not be achieved and we were forced to turn our attention to preparing 9-aminoellipticine instead. The problems attending this work will now be described.

Discussion to Part II

As mentioned in the introductory section we hoped to realize the objectives of the Hantsch hypothesis by preparing a number of ellipticine derivatives via a new route pioneered in this laboratory and observing changes in structure/activity relationships.

In view of the difficulties attendant upon the synthesis of individual molecules the most satisfactory approach would seem to be the preparation of a single derivative which could then be converted into other required structures by relatively simple procedures.

Thus a nitro substituted ellipticine seemed best suited to this purpose since the nitro function may be converted readily into many other functions. Previous work in this laboratory had shown that direct nitration of ellipticines gives only complex mixtures, therefore our initial investigations concerned the preparation of mono-nitroellipticines from the correspondingly substituted 1,3 diacetylindoxyls (65).



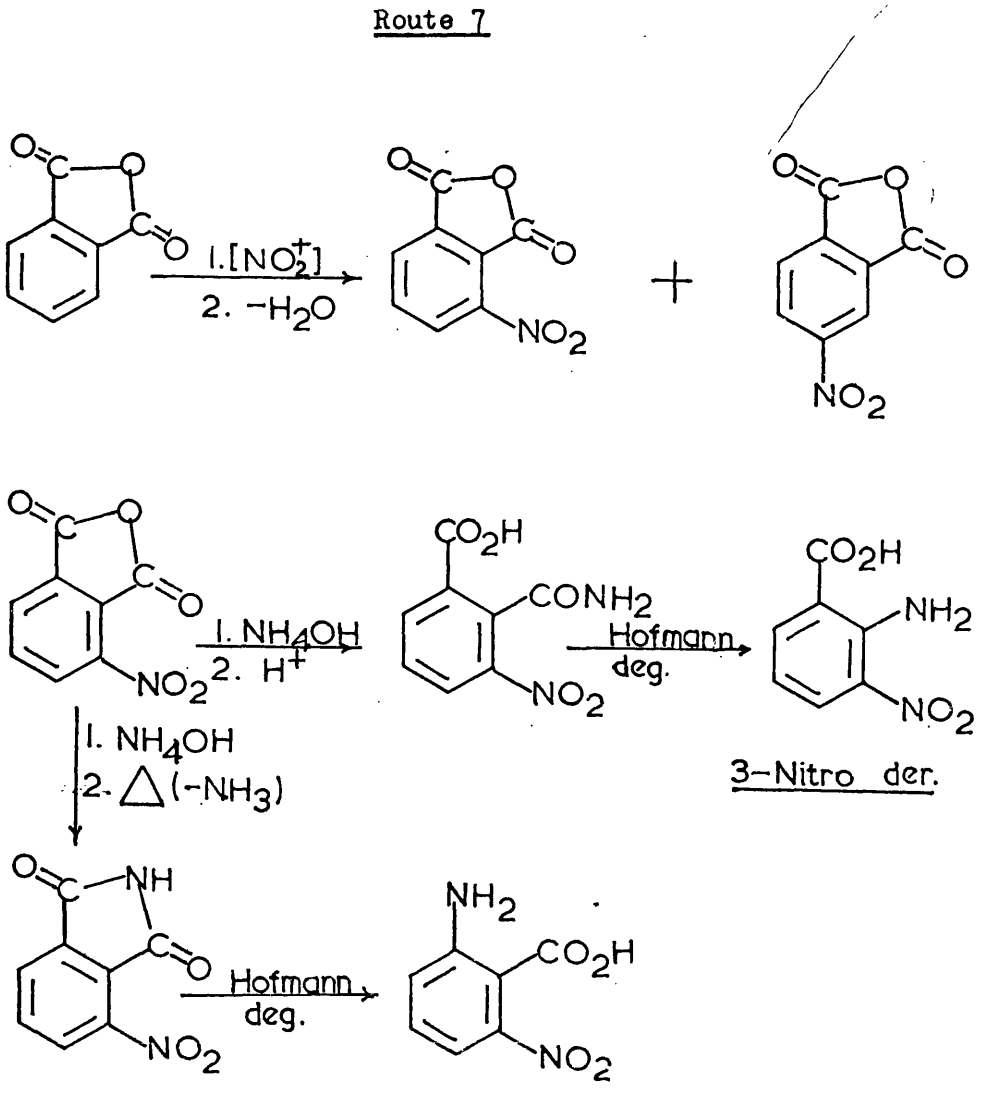
(65)

The preparation of mono-nitro-1,3-diacetylindoxyl

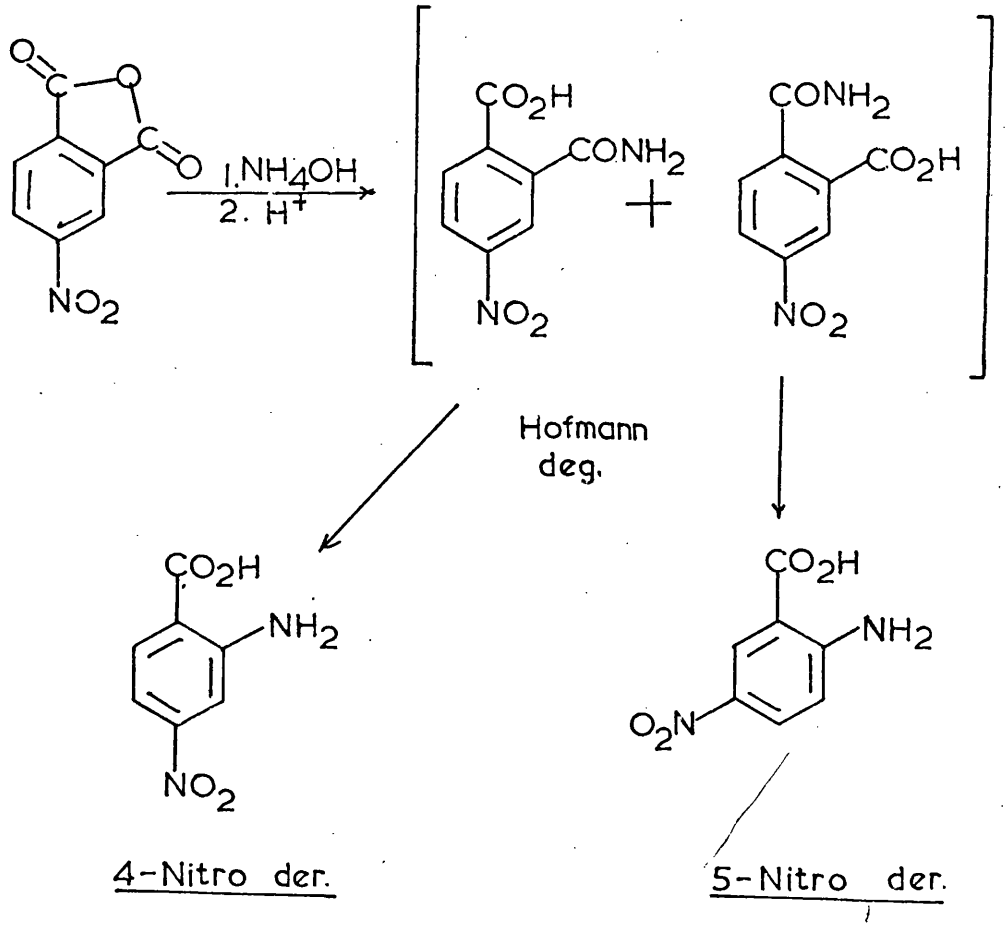
1,3-Diacetylindoxyl may be obtained from anthranilic acid in three stages[80]. This synthesis has general applicability and thus benzenoid substituted indoxyls are easily prepared providing, of course, that the necessary precursors are available.

Although our main objective was the synthesis of 9-nitroellipticine, all four mono-nitroanthranilic acid derivatives may easily be obtained from phthalic anhydride[81] (see Route 7) and thus, potentially, there is access to a wide variety of ellipticines.

Route 7

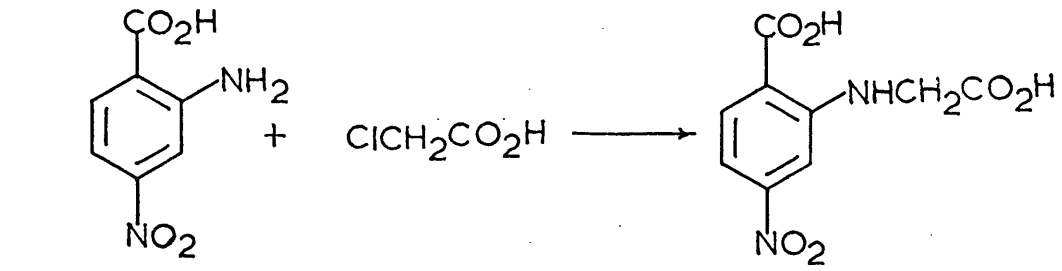


6-Nitro der.

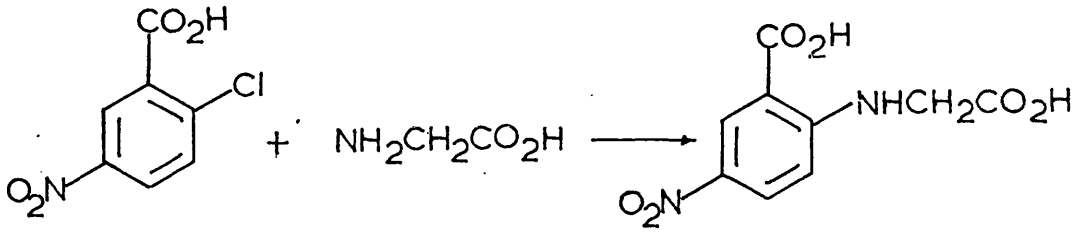


Unfortunately despite repeated efforts we were unable to condense the 3-nitro-isomer with chloroacetic acid. This lack of reactivity of the amino group is not surprising, being probably due to a combination of the steric repulsion and electron withdrawing effects of the adjacent nitro group. Similar problems were encountered with the 6-nitroisomer.

The synthesis of 2-carboxy-5-nitro-phenyl glycine (66) is straight forward[82] but the 4-nitro analogue (67) may not be so prepared (see for example ref. 83), and it is necessary to react 5-nitro-2-chloro benzoic acid (68) with glycine[84].

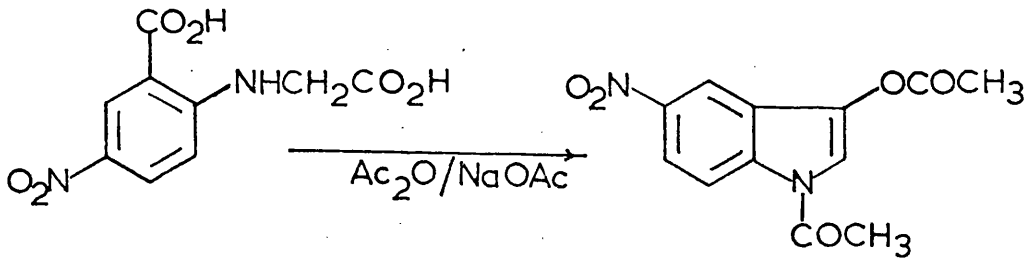


(66)



(68) (67)

The Hantsch proposals deal specifically with 9-substituted ellipticines so that initially our efforts centred around the production of 9-nitroellipticine. With 2-carboxy-4-nitrophenyl glycine (67) in hand it was comparatively simple to convert this into 1,3-diacetyl-5-nitroindoxyl (69) by reaction with acetic anhydride and sodium acetate. Thus one half of the required ellipticine was available and it now remained to prepare 4-acetyl--3-(1-methoxy)ethyl pyridine with which to implement the final stages of route 5 (page 61).

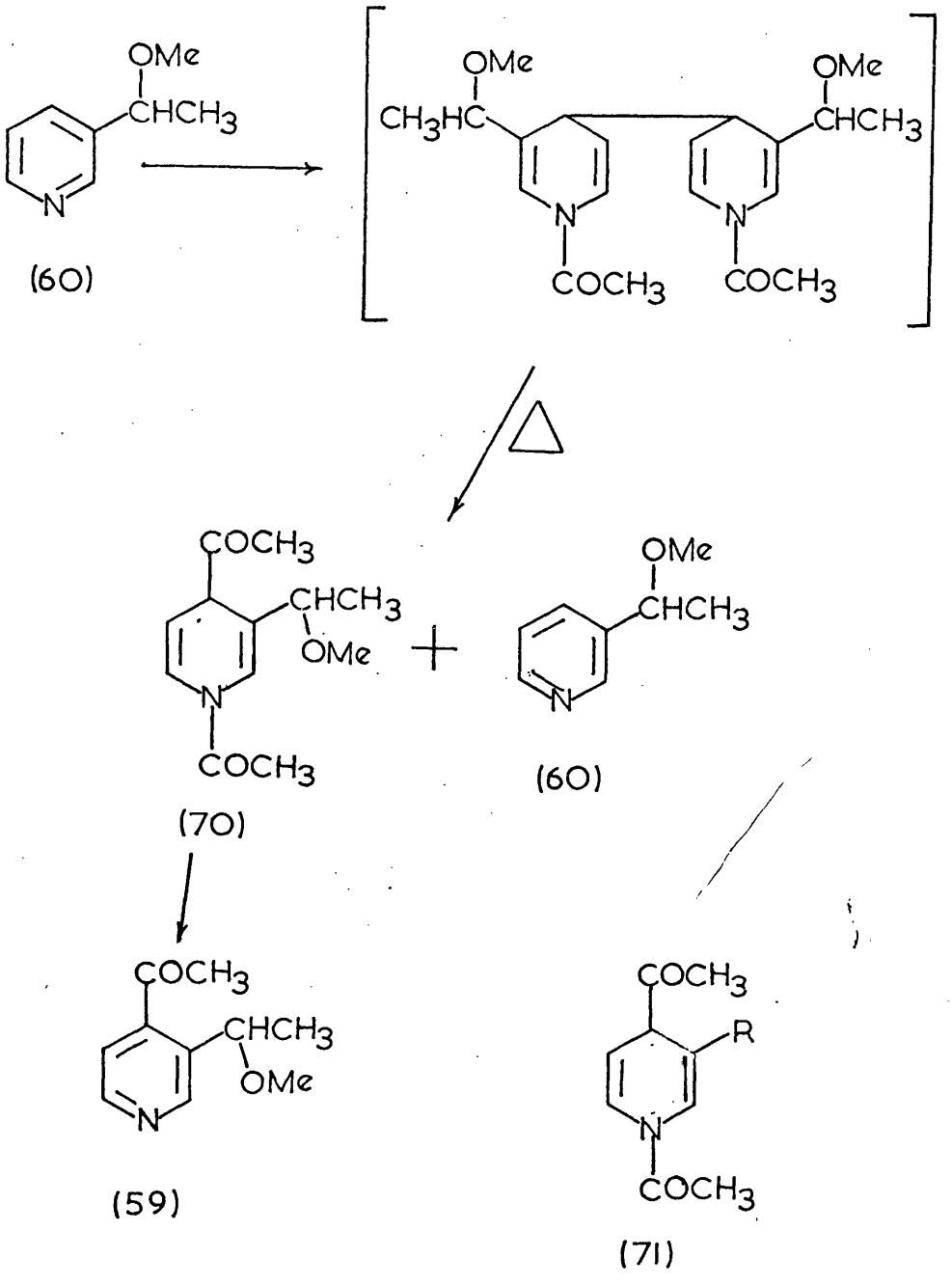


(69)

Preparation of the pyridine component.

Previous studies in this laboratory[77] have shown that it is possible to convert 3(1-methoxy)ethyl pyridine (60) into the 1,4-diacetyl,1,4-dihydro derivative (70) by the so called Wibaut-Arens reductive acetylation technique with acetic anhydride and zinc. Unfortunately this reaction proceeds via a dimeric species which then disproportionates into (70) and starting material. At best therefore the yield of (70) may only be 50% and a further constraint is that the reaction is sensitive to the size of substituents attached to the pyridine ring. Despite the fact that starting material is regenerated it is not easy to separate this from the dihydropyridine which often oxidizes partially to the corresponding pyridine (59).

The steric effect of the 3-substituent is quite marked for although 3-ethylpyridine gives (71, R=Et) in 44% yield we were unable to better a yield of 34% for the methoxy-derivative (70), while (71, R=CH(OAc)CH_3) and (71, R=C(OCH_2CH_2O)CH_3) were not formed under the Wibaut-Arens conditions.

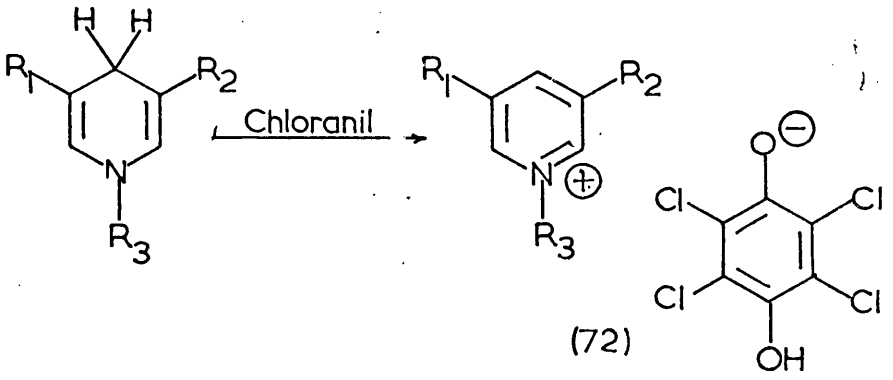


During the several runs necessary to accumulate a sufficient stock of 1,4-dihydro-1,4-acetyl pyridine (70) it became apparent that the success of the reductive acetylation was highly susceptible to small changes in temperature. When zinc dust was added to a solution of the pyridine derivative (60) maintained at a temperature below -5° no reaction occurred, but when the same reaction was allowed to warm to room temperature an exothermic reaction ensued, resulting in the formation of a complex mixture.

The optimum conditions require a temperature of -5-0° during the addition of zinc (4h.) and then stirring at 0-5° for approximately 8h. before allowing to warm up to room temperature.

If the 1,4-dihydro-1,4-diacetyl pyridine (70) is heated in methanol for several hours oxidation to 4-acetyl-3(1-methoxy)ethyl pyridine (59) occurs, however, the yield is low and initially we sought other means of effecting this aromatization. Thus oxidation with chromium trioxide gave a low yield of (59) whereas nitrous acid and iodine failed to give identifiable products.

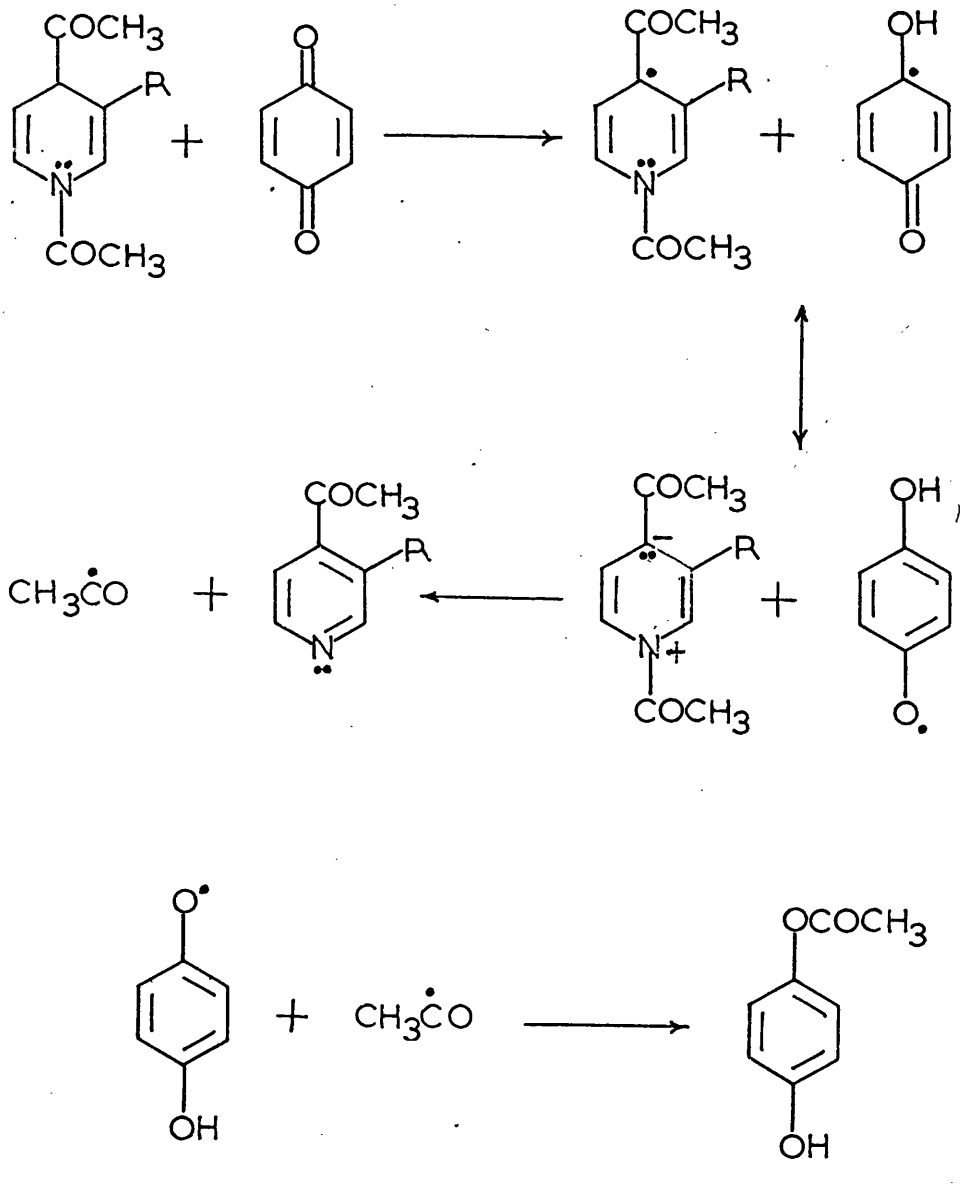
N-Substituted 1-4-dihydropyridine derivatives react with high potential quinones (eg. chloranil) in the manner shown below[85]:



(72)

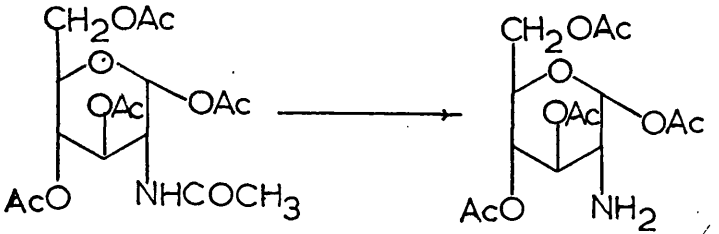
Applied to the substance (70) this reaction might proceed further to give the required product (59) since the corresponding intermediate would probably be unstable and react with any available nucleophile. Indeed partial success was achieved when p-benzoquinone and chloranil were employed in small scale reactions giving yields of (59) in the region of 50-60%. However, attempts to scale up these

reactions reduced the yield considerably. In such cases the major product was a dark coloured resin. Interestingly, however, we also isolated the quinol acetate (73). Presumably this compound arises via a free radical mechanism:



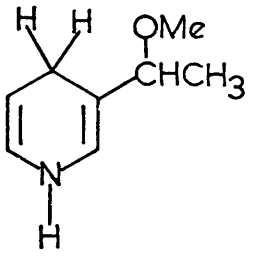
(73)

Meerwein's reagent (triethoxytetrafluoroborate) has been used[86] to achieve selective N-deacetylation of the sensitive sugar (74). If an analogous reaction could be carried out upon (70) aromatization of the product should be straight forward, many such oxidations of the latter type of system are documented in the literature.



(74)

This approach was unsuccessful, however, when applied to the system (70); extensive decomposition occurred and only a trace of a product was obtained. In the mass spectrometer this gave rise to a molecular ion at m/e 149 and thus it may have the structure (75).



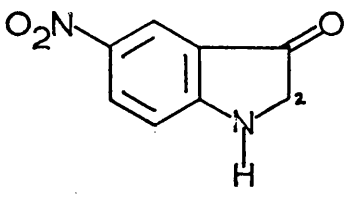
(75)

Because of these failures we reverted to the technique of heating the dihydropyridine in methanol, but unfortunately we found that the yields in scaled up reactions were inconsistent and generally poor. Clearly this reaction involves a radical mechanism and we were able to show that the addition of traces of benzoyl peroxide and ferric chloride improved the overall performance of the reaction.

Nitro-indolinone formation

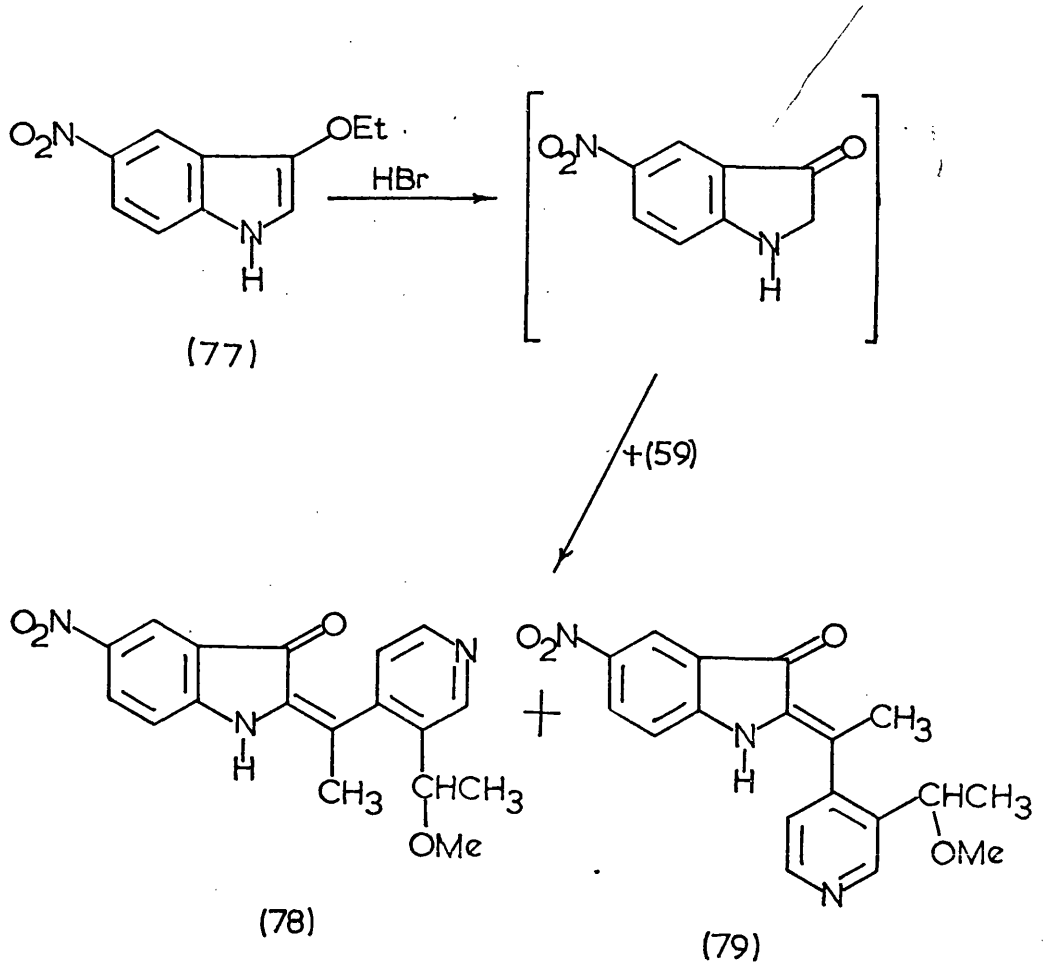
With both intermediates in hand an attempt was now made to condense the two. Disappointingly when a mixture of the indole (69) and the 4-acetylpyridine (59) were reacted in 20% aqueous sodium hydroxide solution a deep brown colour developed and later, when the reaction was worked up, only the ketone (59) was recovered, most of the colour being retained by the aqueous phase.

The reaction between indoxyl and carbonyl compounds depends upon carbanion formation at C-2 in the former; presumably the pseudo-phenolic character of the p-nitrophenylamine unit in (76) reduces deprotonation at C-2 and prevents the desired condensation taking place.



(76)

An acid catalysed reaction, which has been used with success in condensations of indoxyl and various aldehydes[87], seemed an obvious solution to this problem; however, such a reaction between (69) and (59) failed. We suspected that the reason for this failure may have been due to inefficient production of the free indoxyl (76) but when the 1,3-diacetylindoxyl (69) was heated with ethanolic sulphuric acid the ether (77) was obtained. This unexpected product was recognized as a potentially useful intermediate since when heated with aqueous hydrobromic acid in the presence of the ketone the required mixture of geometric isomers (78) and (79) were formed, unfortunately, however, the best yield obtained was only 4%.



The isomers (78) and (79) possess sufficiently different solubility characteristics to allow separation and as expected ^{1}H-n.m.r. spectroscopy shows that each is a mixture of diastereoisomers. This phenomenon derives from the presence of the chiral centre joined to C_3 of the pyridine ring and to the restricted rotation about the bond joining the enone and pyridine units of structure. A clear decision between the diastereomeric pairs is possible since in the case of the (Z)-isomer (79) the methyl group attached to the indole C-2 side chain lies in the deshielding zone of the enone carbonyl group. Thus in the ^{1}H n.m.r. spectrum (Fig. 12) the absorption due to this methyl group occurs at 3.10 whereas in the case of the (E)-isomer (78) this signal is at 2.30. (Fig. 13).

In the (E)-isomer (78) diastereoisomerism causes the same methyl signal to appear as two singlets so that the figure quoted above is the mean position. Similarly two singlets are observed for the O-methyl group; the methine function of the pyridyl side chain at C-3 appears as two quartets and the methyl unit as a double doublet. When the sample is heated, however, these signals merge and it is possible to determine the actual coalescence temperature. From this measurement the potential energy barrier to overcome the restricted rotation about the bond joining the enone and pyridine moieties may be calculated by the first order rate expression[88]:

$$K_r = \frac{KTe}{h} e^{\frac{-Ea}{RT}} \quad \text{.....1}$$

where K = Boltzmann's constant, h = Planck's constant,

T = temperature, Ea = potential energy barrier

and R = gas constant.

The simplified form of the Gutowsky-Holm expression for the line shapes of exchanging species gives:

$$2\tau = \frac{\sqrt{2}}{\pi \Delta \delta}$$

relating the chemical shift difference ($\Delta\delta$) and the lifetime of the state (2τ), at the point where the signals coalesce.

$$K_r = \frac{1}{2\tau} = \frac{\pi \Delta \delta_o}{\sqrt{2}}$$

(where $\Delta\delta_o$ is the chemical shift difference in Hertz at 273°K).

Substituting into equation 1 gives

$$\frac{\pi \Delta \delta_o}{\sqrt{2}} = \frac{KT_c}{h} \cdot e^{\frac{-Ea}{RTc}}$$ (where Tc = coalescence temperature)

therefore $$e^{\frac{-Ea}{RTc}} = \frac{h\pi \Delta \delta_o}{\sqrt{2}\, KT_c}$$

$$\frac{-Ea}{RTc} = \log_e \frac{h\pi}{2K} + \log_e e \frac{\Delta \delta_o}{Tc}$$

$$Ea = RTc \left[\log_e \frac{\sqrt{2}K}{h\pi} + \log_e \frac{\Delta \delta_o}{Tc} \right]$$

$$Ea = 2.303 \times R \times Tc \left[\log_{10} \frac{\sqrt{2}\,K}{h\pi} + \log_{10} \frac{Tc}{\Delta \delta_o} \right]$$

$$Ea = 4.59\ Tc \left[9.97 + \log_{10} \frac{Tc}{\Delta \delta_o} \right] \quad \ldots\ldots\ldots\ldots\ldots 2$$

Thus to evaluate Ea a graph of chemical shift difference ($\Delta\delta$) vs temperature (°K) was constructed (Fig. 14) and from it, values of Tc (coalescence temperature) and $\Delta\delta_o$ (chemical shift difference at 273°K) were measured.

^{o}K	$\Delta\delta$ for -OMe function
303	9.2
323	8.0
333	6.6
343	4.0

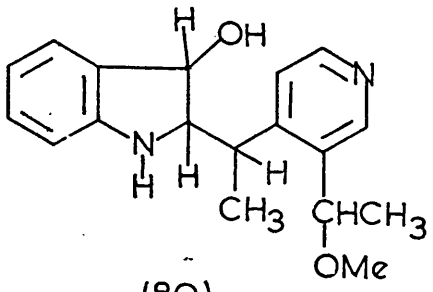
From the graph (Fig. 14) $T_c = 349^{o}$ and $\Delta S_o = 9.6Hz$ and therefore:

$$\underline{E_a = 77.2 \text{ KJ/mole}}$$

In the case of the (Z)-isomer (79) the coalescense temperature lies close to the normal running temperature of the n.m.r. spectrometer and in consequence Ea was not calculated.

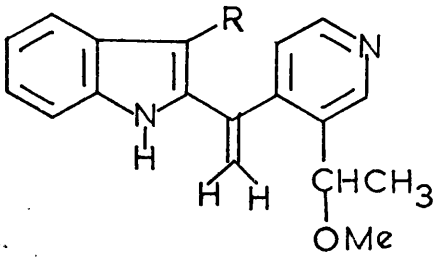
Returning to the projected synthesis the product yield in the condensation reaction between the indoxyl derivative and the 4-acetylpyridine derivative was too low to allow further progress and it became necessary to consider alternative routes.

One possibility was the direct nitration of the indole (64) and to initiate this investigation the indolinones (62) and (63) were prepared and the mixture reduced with sodium borohydride in ethanol solution. The intermediate alcohol (80) was not isolated, but dehydrated to (64) by acid treatment (see Route 5, page 61). Yields were normally high.



(80)

On one occasion after sodium borohydride treatment the solvent ethanol was removed and the residue partitioned between chloroform and water. The organic phase was deep blue-violet in colour and when this was treated with acid a red gum resulted. Trituration of this with ethanol caused partial crystallization of the required indole (64), but from the mother-liquor a quantity of the vinyl derivative (81, R=H) was obtained.



(81)

An interesting feature of the ^{1}H-n.m.r. spectrum (Fig. 15) of this latter molecule is that the methylene unit of structure gives rise to two singlets at 5.13 p.p.m. and 5.80 implying non-equivalence, however, there is no obvious coupling between the two signals. A similar phenomenon is apparent in the spectrum of the O-acetate

(81, R = OAc) formed by the action of acetic anhydride and triethylamine on either (62) or (63)[89].

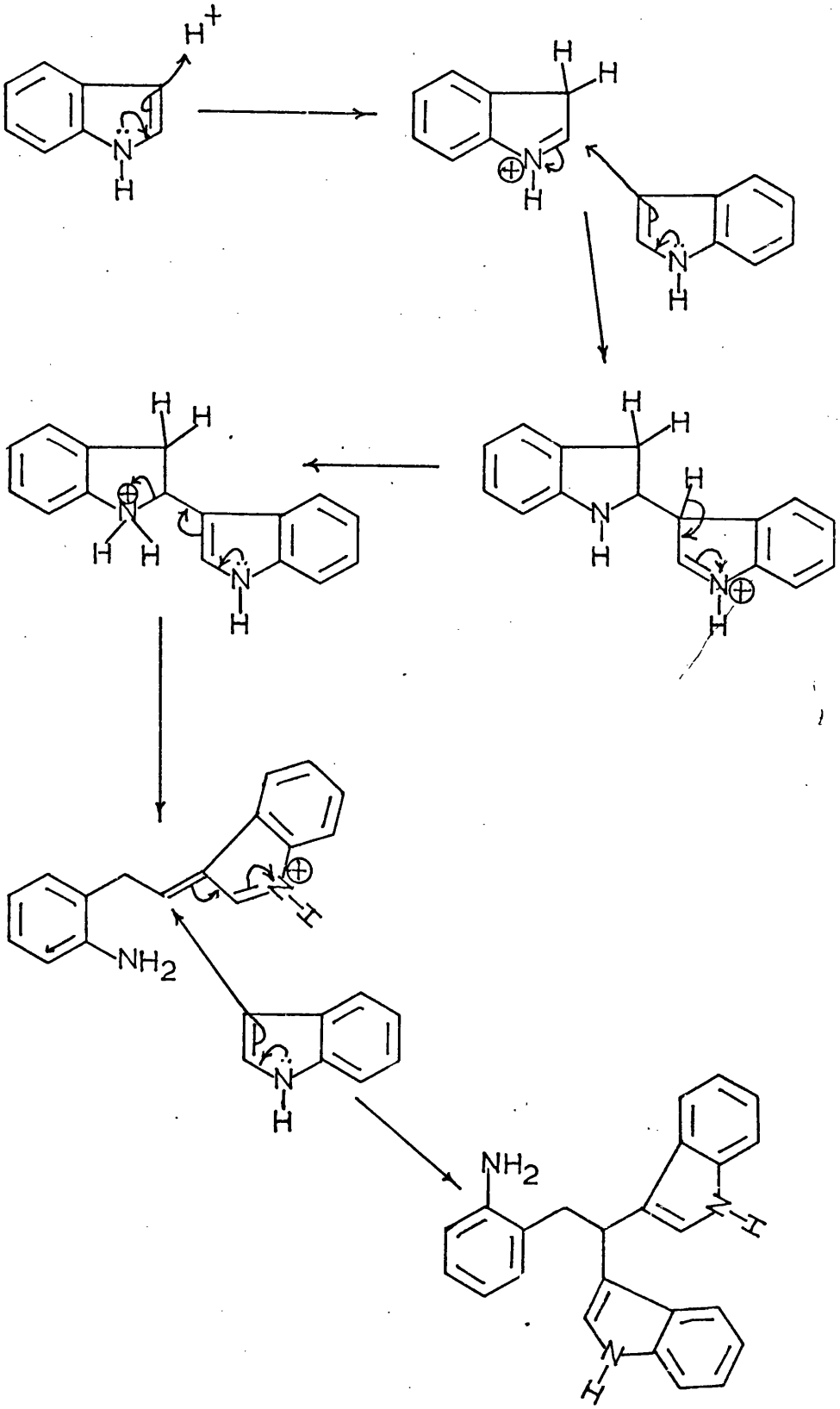
The formation of the vinyl compound (81, R = H) presents ar interesting problem which will be discussed later (page 93)

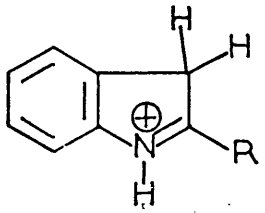
Nitration of the indole (64)

Prior to the actual nitration of the indole (64) a review of the nitration reactions of 2-alkyl indoles was conducted in order to ascertain the probable site of substitution under various conditions.

Indoles unsubstituted at C-2 polymerize under normal nitrating conditions due to initial C-3 protonation and subsequent attack by other indole molecules[90] (see Route 8) 2-Alkyl-indoles, however, may be nitrated in acid solution and it is generally accepted that steric factors and electron donation from the substituent group stabilize the conjugate acid (82) enough to prevent polymerization.

Route 8





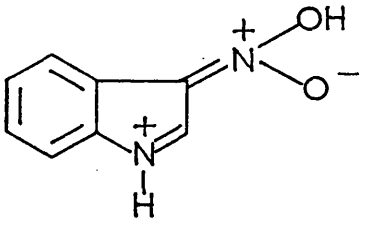
(82)

The actual position entered by the nitro group (s) depends on the conditions, thus in warm concentrated nitric acid alone, or diluted with glacial acetic acid 2-alkyl indoles yield the corresponding 3,6-dinitro derivatives[91]. When the conditions are more forcing 3,4,6-trinitro indoles form. Noland and co-workers[91] propose that in these media the nitrosonium ion (NO^+) is the initial electrophile, which attacks at C-3 the small amount of free indole in equilibrium with its conjugate acid. These authors noted that usually an induction period is necessary and when the reaction finally takes place large amounts of nitric oxide are evolved.

Noland's group propose that once formed, the 3-nitrosoindole undergoes oxidation with nitric acid to form the 3-nitro-analogue and more nitrosating agent. The delay prior to reaction is

necessary in order that sufficient nitrosonium ion is accumulated to sustain reaction and in support of this it may be shown that if sodium nitrite is added no induction period is required.

Noland considers that the presence of a 3-nitro function much reduces the basicity of the indole so that conjugate acid formation is disfavoured and nitration then proceeds in the benzenoid ring at position C-6. Further attack may occur at C-4. The validity of Noland's structural assignments are verified by synthetic studies, but his conclusions seem rather obscure since if the free indole is the substrate, attack by the electrophile para to the indole N-atom might be anticipated. On the other hand if one considers the conjugate acid (83) attack at the 6 and 4-positions (meta to the positively charged N-atom) would be unexceptional.

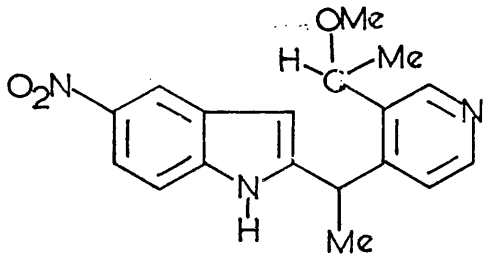


(83)

When 2-substituted indoles are nitrated in concentrated sulphuric acid by the addition of sodium nitrate, 5-nitroindole derivatives are produced[92]. Nitration at C-3 does not normally occur and 5,7-dinitro products have not been reported.

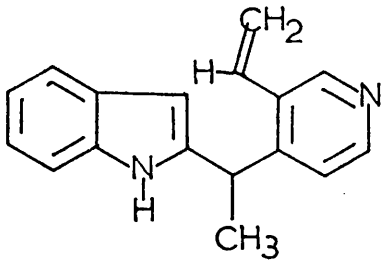
N.m.r. and u.v. spectral studies[93] on various methyl substituted indoles show that in concentrated sulphuric acid solution they exist almost entirely as the conjugate acid. This fact is taken to explain the lack of reactivity at C-3 towards the nitronium ion and Noland regards the imminium bond to be capable of <u>para</u> direction, but once again it is not easy to accept this view point. Nevertheless synthetic studies seem to confirm his orientation assignments.

Our requirement was the formation of the 5-nitroindole (84) so we considered that concentrated sulphuric acid and sodium nitrate were the reagents of choice, but before committing our available material we decided to examine the effect of concentrated sulphuric acid upon a small sample.



(84)

At temperatures ranging from 0-90° the parent indole was regenerated unchanged, but, after heating at 100°, progressively less of the indole was liberated upon basification as the duration of the experiment was extended and after 30 minutes, the only product isolated was the 3-vinylpyridine derivative (85).



(85)

Satisified that the indole (64) was relatively stable we next attempted a nitration experiment using ice cold concentrated sulphuric acid to which potassium nitrate (4 molar equivalents) was gradually added. On work-up a red-orange gum was obtained, this on trituration with ether afforded a yellow solid which was crystallized from ethanol.

Elemental analysis indicated the molecular formula $C_{18}H_{17}N_5O_7$, i.e. a trinitro derivative. In the mass spectrum the expected molecular ion peak m/e 415 is of very low intensity.

Since trinitration under these conditions has not been reported previously it was of some interest to determine the orientation pattern of this compound.

The ^{1}H n.m.r. spectrum (Fig. 16) in $(CD_3)_2SO$ is summarized below:

1.0 p.p.m., 3H, triplet (J = 7Hz), $\underline{CH_3}.CH_2.OH$

1.4 p.p.m., 3H, doublet (J = 6.5 Hz), $\underline{CH_3}.CH(OMe)$

1.17 p.p.m., 3H, doublet (J = 6.5 Hz), $\underline{CH_3}.C$

3.1 p.p.m., 3H, singlet, OMe

3.5 p.p.m., 2H, quartet (J = 7Hz), $CH_3.\underline{CH_2}.OH$

4.8 p.p.m., 1H, quartet (J = 6.5 Hz), $\underline{CH}(OMe)CH_3$

5.5 p.p.m., 1H, quartet (J = 6.5 Hz), $\underline{CH}.CH_3$

6.0–6.5 p.p.m., 3H, v.broad singlet*

7.0 p.p.m., 1H, doublet (J = 5Hz), 5' - H

8.35 p.p.m., 1H, broad singlet, benzenoid proton

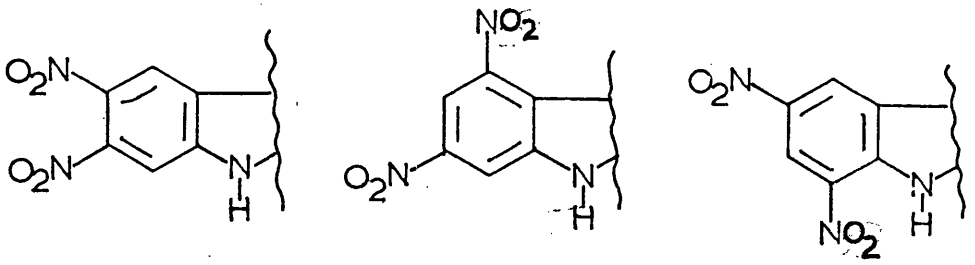
8.5 p.p.m., 1H, broad doublet 6' - H

8.7 p.p.m., 2H, broad singlet, 2'-H and benzenoid proton.

From this data it is clear that two nitro groups are present in the benzenoid ring and the other must be at C-3 since the signal anticipated for the proton at this position (δ ca 6.5 p.p.m. in $(CD_3)_2SO$) is absent.

The two benzene proton resonances occur at δ 8.35 and δ 8.7 as broad singlets, thus clear distinction between the orientations A, B or C is not possible.

* Active protons, lost on treatment with D_2O



(A) (B) (C)

In order to provide a structural assignment we compared the electronic absorption spectrum of our product with those of variously substituted nitroindoles* viz:-

2-Methyl-3,5,6-trinitro[91]: $\lambda_{max}(\varepsilon)$(conc. 3.682×10^{-5}M) 230 (13,180), 244(13,180), 317(11,220) and 386(8,318)nm

2-Methyl-3,5,7-trinitro[91]: $\lambda_{max}(\varepsilon)$(conc. 3.056×10^{-5}M) 284(17,780) and 350(9,550)nm

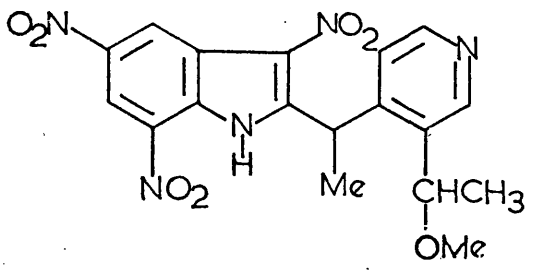
3,5,7-trinitro[94]: $\lambda_{max}(\varepsilon)$(conc. 4.12×10^{-5}M) 216(19,950) 286(12,900) and 413(2,344)nm

2-Methyl-3,4,6-trinitro[91]: $\lambda_{max}(\varepsilon)$(conc. 2.539×10^{-5}M) 294(15,850) and 347(12,020)

Our product: $\lambda_{max}(\varepsilon)$($2.46\times10^{-5}$M) 272(17,800), 330(11,500) and 411(10,200)nm.

Unfortunately an unequivocal decision is not possible although the correlation between our compound and 2-methyl-3,5,7-trinitro indole is reasonably close. In consequence we tentatively assign structure (86) to it.

* The concentration has been given since these substances do not obey Beer's law.

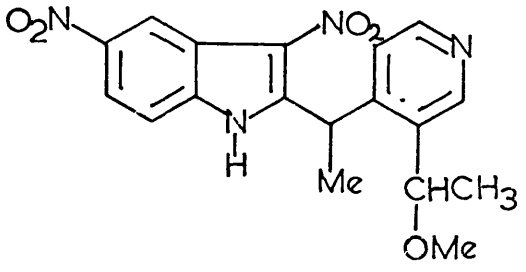


(86)

In support of this conclusion when the nitration reaction was repeated, this time using only one molecular equivalent of potassium nitrate, a dinitro compound was isolated.

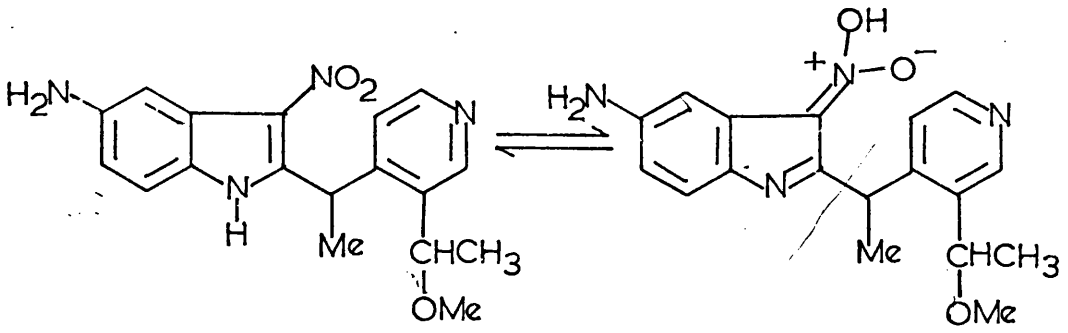
The 'H n.m.r. spectrum of this product is reproduced in figure (17). Unlike the spectrum of the trinitro-product clear evidence of diastereoisomerism is shown and the ABX splitting pattern of the three benzenoid protons indicate nitro substitution to be at either C-5 or C-6.

Figure (18) illustrates the electronic absorption spectrum of this compound in relation to those of 3,5-dinitro-2-methyl and 3,6-dinitro-2-methyl indoles. From this it would seem that the structure is probably (87).



(87)

Interestingly, when the dinitro-compound was hydrogenated in ethanol solution only one of the nitro functions was reduced. In the infra-red spectrum (nujol) this mono amino product showed major bands at 3410 and 3320 ($-NH_2$) and 1630cm^{-1}. This last fact and the virtual absence of an absorption expected for a nitro group ($\sim$ 1530cm^{-1}) suggests that although the nitro group is formally sited at C-3 the compound may exist in the oximino form (88) in the solid state.



(88)

If the results of our experiments are correctly interpreted they represent a radical departure from normal experience, thus here we find nitration at C-3, polynitration and, moreover, an unexpected orientation of substituents.

We can only offer a tentative rationalization of our results, but because of the pressure to complete our major objectives we were unable to provide evidence in support of it. We suggest that in concentrated sulphuric acid the indole undergoes substitution in the benzenoid ring (at C-5) first. This event and the presence of the pyridinium cation reduces the stability of the conjugate acid so that

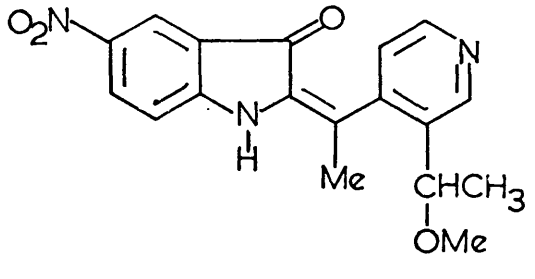
additional nitration may occur at C-3. The formation of the trinitro product may be more easy than expected at first sight; for example, because the two nitro groups at C-3 and C-5 are cross conjugated with respect to the indole N-lone pair electrons, the latter are more available for stabilization of the transition state required for C-7 substitution.

From these experiments it seemed that an approach based on the direct nitration of the indole was unprofitable and so we directed our attention towards using the indolinones (62) and (63) as the substrates for nitration.

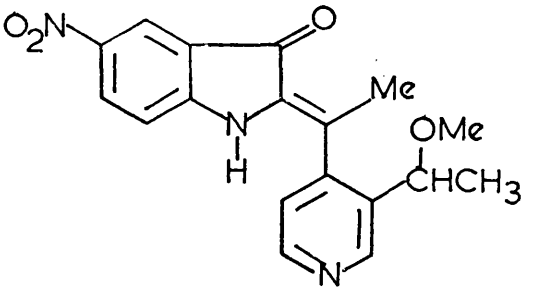
Nitration of the indolinones.

Just as indigotin has been observed to undergo nitration in water-free systems[95,96], we hoped the indolinone mixture (62) and (63) would react in concentrated sulphuric acid solution.

In practice, however, we find that only moderate yields of the mono-nitroindolinones (78) and (79) are obtained, these products being accompanied by much intractible tar.

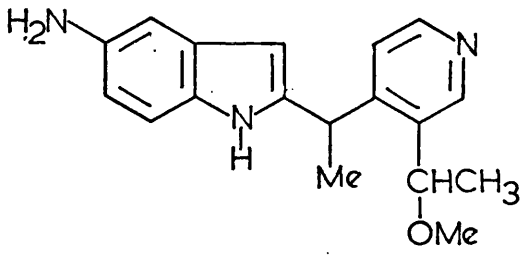


(78)



(79)

A mixture of the nitroindolinones was subjected to sodium borohydride reduction in boiling ethanol and then without characterization of the intermediate alcohol the product was dehydrated by treatment with hydrogen chloride in methanol. However, this procedure did not give the expected indole (84), but rather a small quantity of a white solid, suspected from mass spectral data (m/e 295, 248 (base) and 233) to be the 5-amino indole (89).

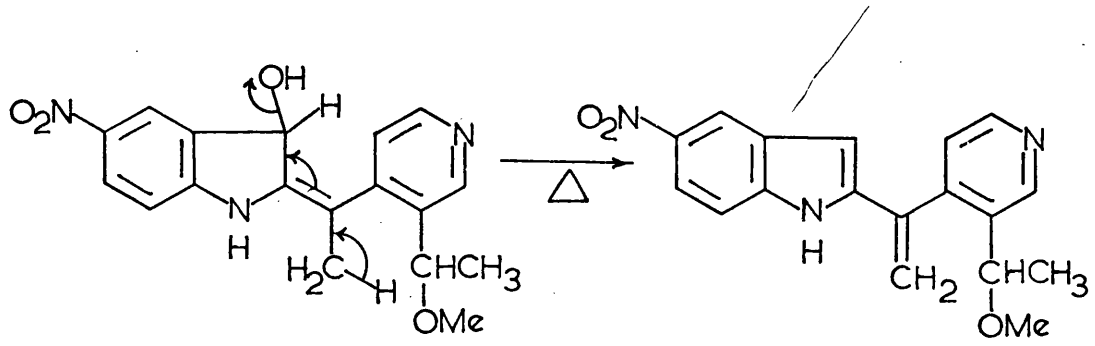


(89)

Proof of this structural assignment was achieved later when the acetyl derivative of this product was shown to be identical with an authentic specimen prepared by another route (see page 99). Although this result was a disappointment to us it is of interest to note that nitro groups are not normally affected by sodium borohydride under these conditions. The reason for the susceptibility of the nitro group in this case is not clear.

Repetition of the reduction at room-temperature yielded an unstable substance, which upon crystallization from ethanol gave red coloured prisms as the first crop and then a yellow solid.

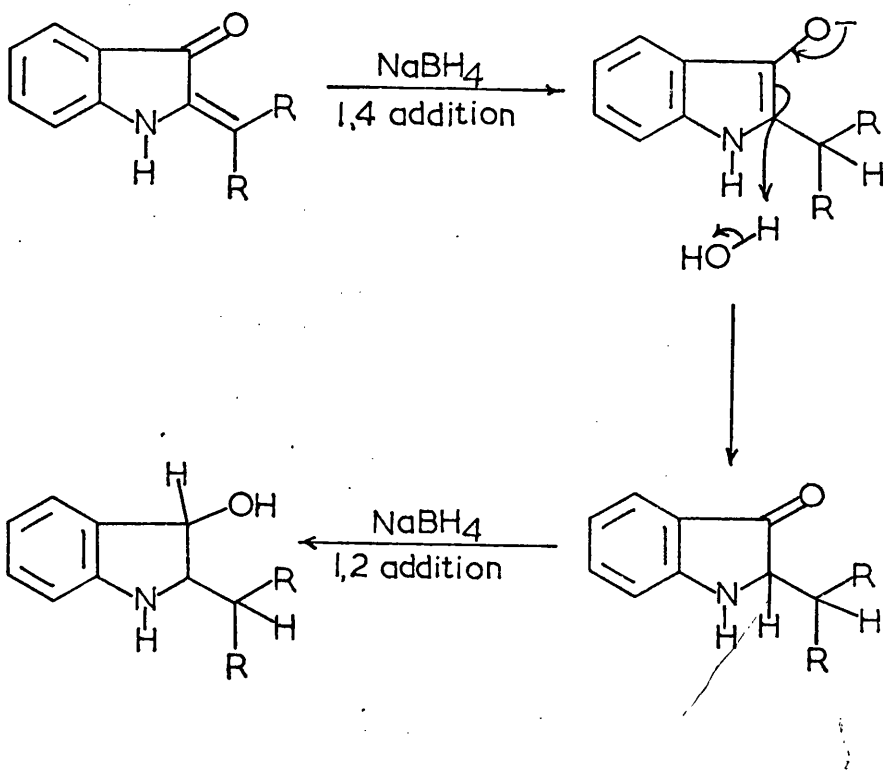
The red crystals were obviously impure, but mass spectroscopy showed a molecular ion at m/e 341 which readily lost 18 mass units ($-H_2O$). In the infra red spectrum a broad band 3600-3200 cm^{-1} indicated an alcohol, while absorption maxima at 1510 and 1330 cm^{-1} revealed that a nitro group was present. These facts, together with the ^1H n.m.r. date (see experimental section), suggest that this product is (90) and this conclusion is supported since on heating in benzene in a Dean Stark apparatus, the compound readily dehydrated to the vinyl indole (91) (cf (81) page 80) which was yellow in colour.



(90) (91)

Mechanistically, the formation of (91) is in conflict with the work of Hooper and Pitkethly[87] who have shown that, normally reduction of the α-β-unsaturated system of alkylidene-indolinones with sodium borohydride involves 1,4-addition of hydrogen as the first step (see route 9).

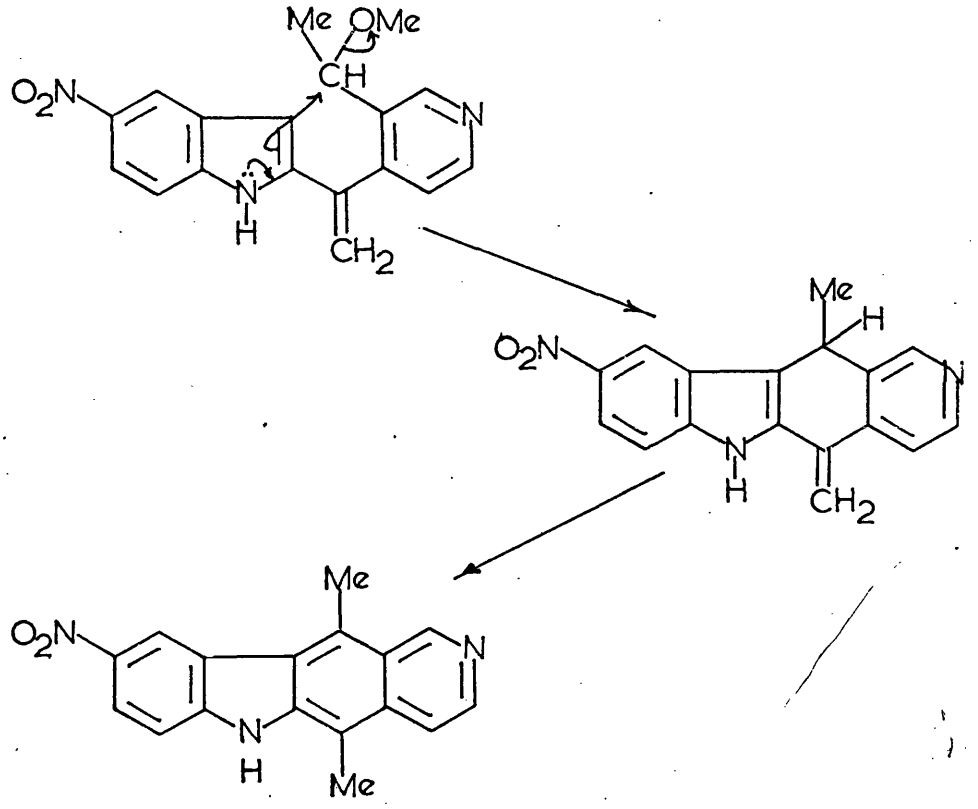
Route 9



Unless tautomeric phenomena are evolved the formation of the vinyl indole (91) must occur by initial 1,2-addition of hydrogen to the carbonyl function of (78) and (79) generating the respective alcohol e.g. (90) from the (E)-isomer which on heating dehydrates to (91).

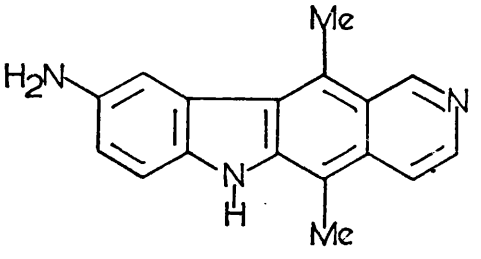
With (91) in hand we presumed that ring closure to ellipticine should be relatively simple since both compounds are at the same oxidation level, the reaction only requiring the formal loss of MeOH. Tautomerism to the fully aromatic tetracyclic pyridocarbazole system should be spontaneous (see Route 10).

Route 10



9-Nitroellipticine

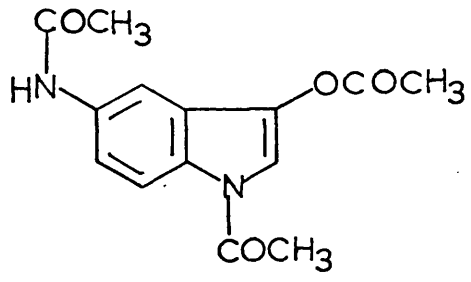
An attempt to bring about this reaction by heating (91) in aqueous hydrobromic acid (60%) failed. Regretfully therefore, we decided to abandon the projected synthesis and turn our attention to the synthesis of 9-aminoellipticine (92).



(92)

The Synthesis of 9-Aminoellipticine

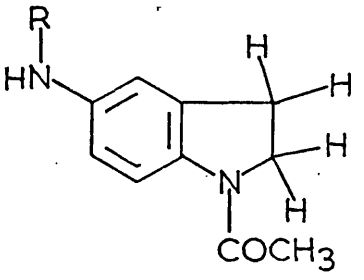
The previous results show that the nitro-indole (91) may be synthesised from the indolinones (62) and (63) and presumably hydrogenation of (91) would give the amino-indole (89). By treating with hydrobromic acid this may then give 9-aminoellipticine; however, in view of the poor yields already encountered we did not think this route was worth pursuing. We therefore returned to the basic scheme (route 5, page 61) and hoped to employ the 5-acetamido derivative (93) as the indolinone precursor.



(93)

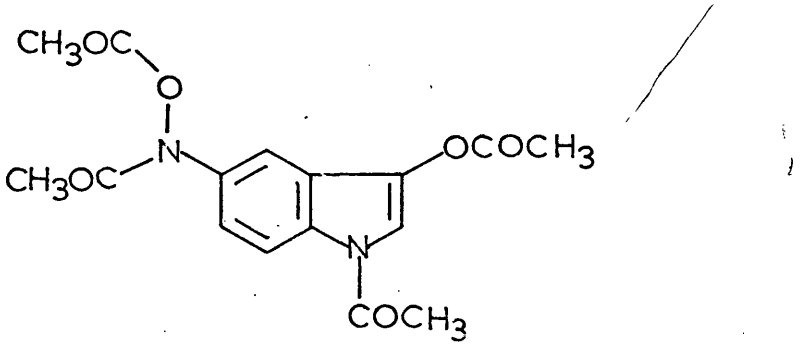
Hydrogenation of 5-nitro-1,3-diacetylindoxyl

When a solution of (69) in glacial acetic acid was hydrogenated over Adam's catalyst at atmospheric pressure the amino indoline (94, R = H) was formed and when acetic anhydride was present the derivative (94, R = Ac) was produced.



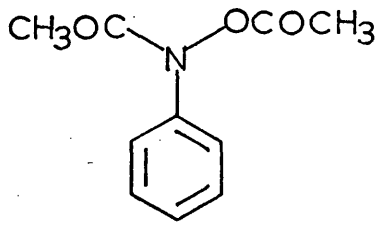
(94)

By using a mixture of acetic anhydride and dimethyl formamide as solvent and 5% Pd on carbon as catalyst the heterocyclic system remained intact, but instead of the required 5-acetamido-1,3-diacetyl indoxyl we obtained the substance (95).



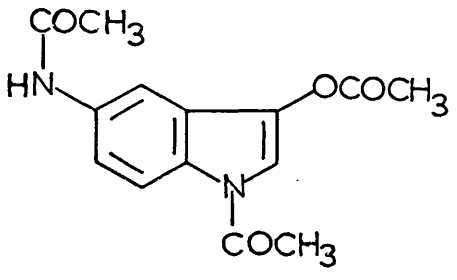
(95)

A band at 1785 cm^{-1} in the IR spectrum was assigned to the $NOCOCH_3$ function and this compares well with the spectral properties of (96), prepared by acetylating phenyl hydroxylamine.

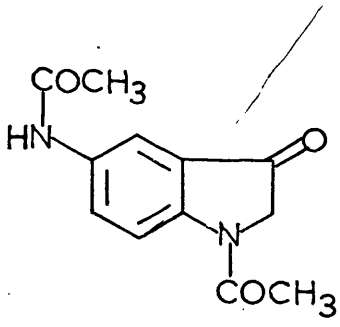


(96)

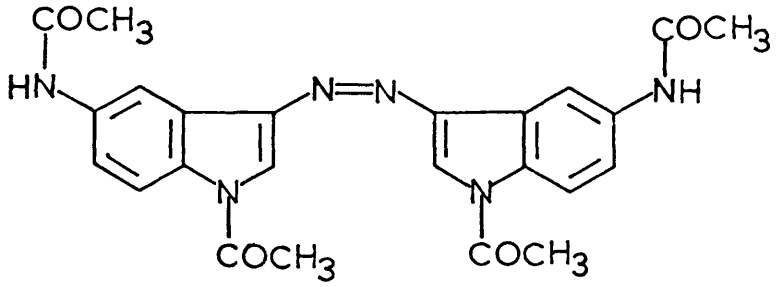
Finally when the acetylation was performed in a separate step after hydrogenation in dimethyl formamide solution alone we obtained (93) together with small quantities of (97) and (98).



(93)

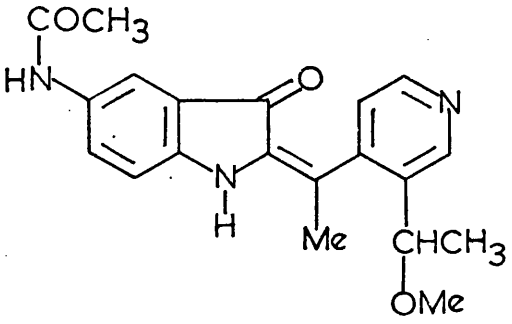


(98)



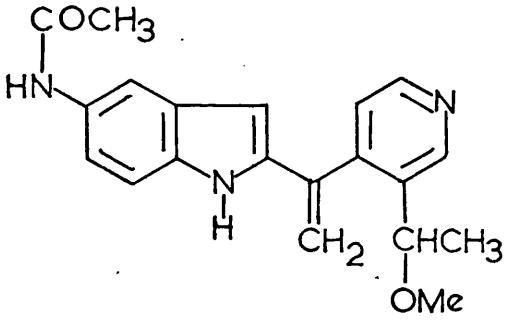
(97)

The 5-acetamidoindoxyl (93) was condensed with the 4-acetylpyridine (59) and though generally the product failed to crystallize from the reaction mixture, on one occasion deep red prisms separated. The ^{1}H n.m.r. spectrum (Fig. 19) indicated this to be the (E)-isomer (99) since the absorption due to the olefinic methyl appeared at δ 2.15 p.p.m.

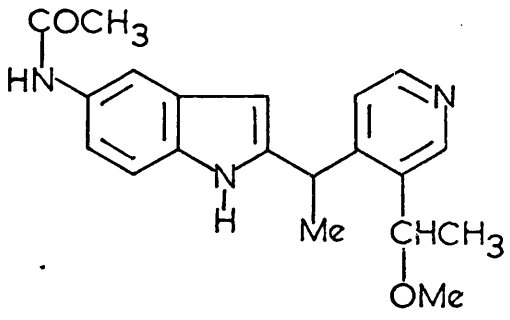


(99)

Sodium borohydride reduction of (99) in refluxing ethanolic solution, followed by dehydration, presented no problems except that when insufficient reducing agent was employed the vinyl indole (100) contaminated the product (101).



(100)

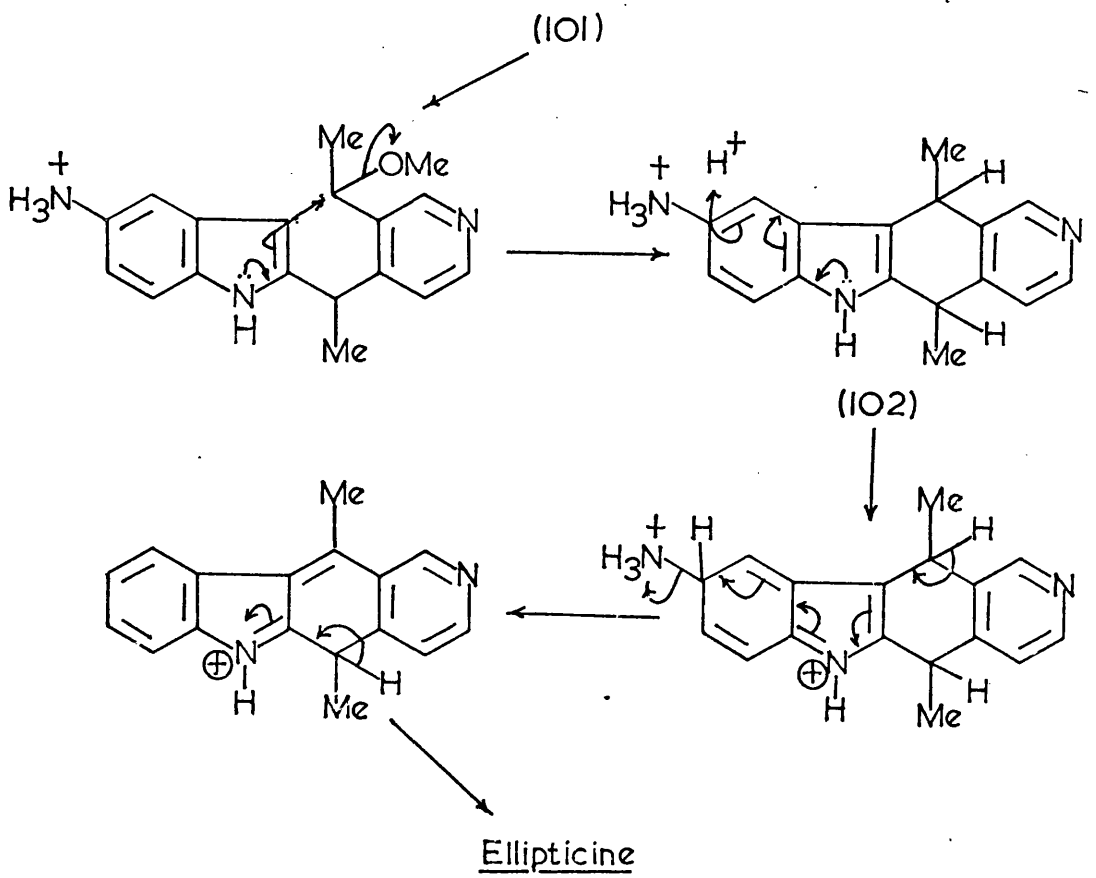


(101)

The final stage of the synthesis was effected by heating a solution of the indole (101) under reflux in aqueous hydrobromic acid (60%) for approximately 18h., during which time a yellow deposit collected. Thus by filtering the strongly acid solution through sintered glass the dihydrobromide salt of 9-aminoellipticine (92) was obtained and on treatment with sodium hydrogen carbonate the free base was liberated in 38% yield. On working up the mother-liquor we were intrigued to discover that this consisted mainly of unsubstituted ellipticine.

The production of ellipticine in this reaction is interesting since it must involve a reductive mechanism and in the absence of added reducing reagents probably involves the dihydro intermediate (102). Route 11 offers a possible explanation of this result.

Route 11



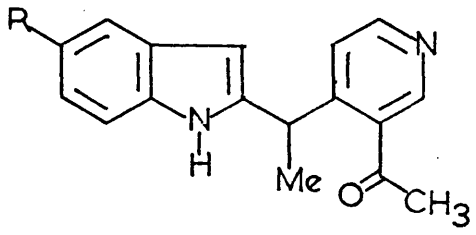
Although we had achieved a synthesis of 9-aminoellipticine the overall yield was very poor and provided only sufficient material for evaluation of its activity against the leukemic mouse (L 1210). As our main objective was to use this compound as a starting point for the synthesis of several other derivatives we deemed it essential to devise a better syntheses of 9-aminoellipticine.

AN IMPROVED SYNTHESIS OF ELLIPTICINE DERIVATIVES

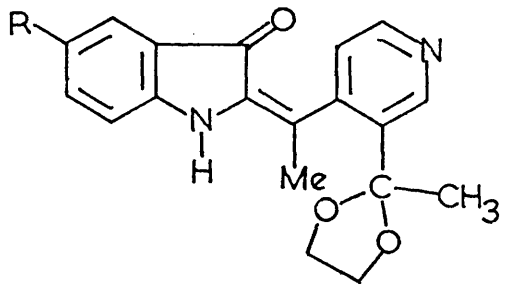
Introduction

The low overall yield of 9-aminoellipticine obtained by route 5 stems firstly from an inefficient synthesis of the 3-methoxyethyl--pyridine (59) and secondly the severe conditions necessary to effect ring closure of (101) cause appreciable loss of the final product by deamination (see route 11). Therefore any new synthesis based on route 5 requires the replacement of the pyridine (59) by a more accessible alternative which in turn gives rise to a derivative more easily cyclized to the pyridocarbazole system.

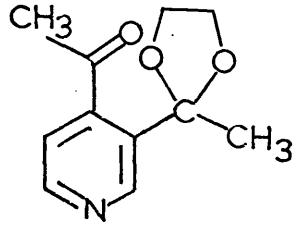
Indoles readily undergo electrophilic attack by carbonyl compounds in mildly acid media[96] and therefore our primary objective was an efficient synthesis of the indole (103). To achieve a synthesis of (103) we considered that the intermediate (104) or its stereo isomer would be most useful and consequently a synthesis of the pyridine (105) was investigated, this with the appropriate indoxyl should form (104).



(103)



(104)

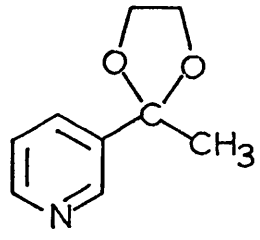


(105)

Synthesis of 3,4-diacetylpyridine and derivatives

(i) By free radical means

Initially it was hoped that the 3,4-diacetylpyridine derivative (105) might be prepared by the Wibaut-Aren's reductive acetylation procedure using the acetal (106). Unfortunately all attempts failed and only starting material was isolated from the reaction products.

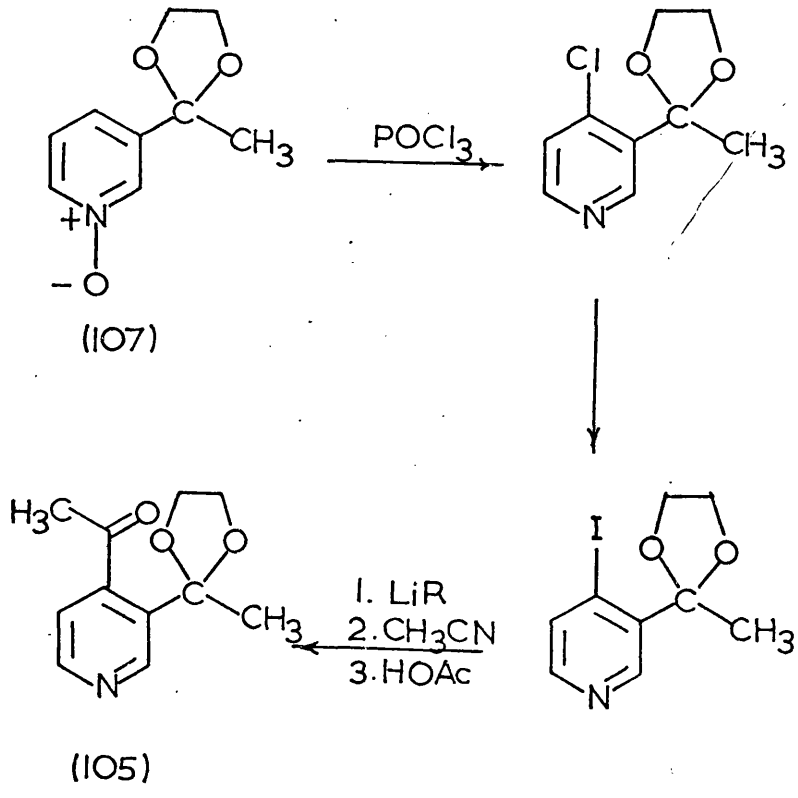


(106)

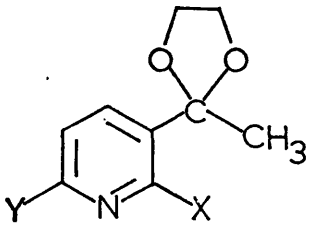
(ii) By electrophilic attack

Unlike 3-methylpyridine itself, 3-methylpyridine-N-oxide undergoes halogenation at C-4 when treated with phosphorus oxychloride[97] and although 4-chloropyridines are inactive towards lithylation[98] this is not so for the 4-iodo analogues. As it is known[99] that chloropyridines may be converted into iodopyridines we envisaged a synthesis of (105) via route 12.

Route 12.

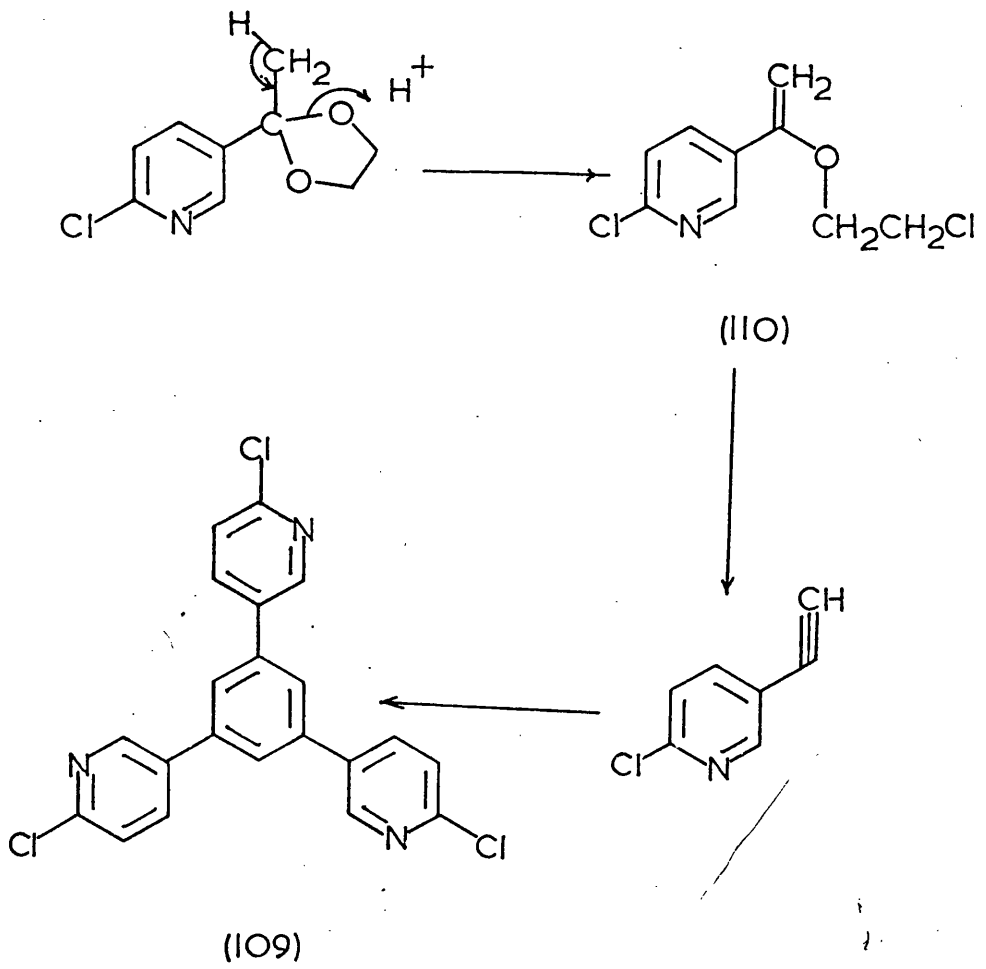


Thus the pyridine-N-oxide (107) was prepared and reacted with phosphorus oxychloride; the crude product was shown to contain the 2-chloropyridine (108, X = Cl, Y = H) which from an analysis of the ^{1}H-n.m.r. spectrum was contaminated with approximately 5% of the 6-chloroisomer (108, X = H, Y = Cl). No evidence for the anticipated 4-chloro derivative was obtained.



(108)

In addition to these monochloro products, a pale yellow compound (m.p. 264-6°) was obtained; from mass spectrometric analysis this gave rise to a molecular ion cluster at m/e 411/413/415. In the ^{1}H-n.m.r. spectrum (Fig. 20) only aromatic proton signals were observed, grouped into a singlet and an ABX splitting pattern. From this evidence, together with elemental analysis, the product is considered to be the tetracycle (109) and a possible mechanism for its formation is indicated below:



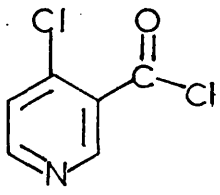
(110)

(109)

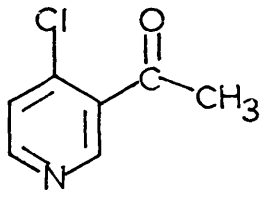
Indeed, we have some circumstantial evidence for this pathway since the crude tetracycle is contaminated with traces of a component which in the mass spectrometer gives rise to a molecular ion cluster at m/e 217/219/221. This may correspond with the intermediate (110).

Clearly the above approach to (105) is inapplicable and next the known[100] acid chloride (111) was prepared as the hydrochloride salt and reacted with excess dimethyl cadmium.

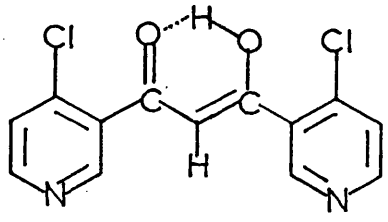
In this way it was hoped to obtain 3-acetyl-4-chloropyridine, but the only product isolated was the stable enol (112) and, although the structure of this compound suggests that some of the ketone (113) is generated during this reaction, a number of modifications to the conditions failed to provide an efficient route to this compound.



(111)

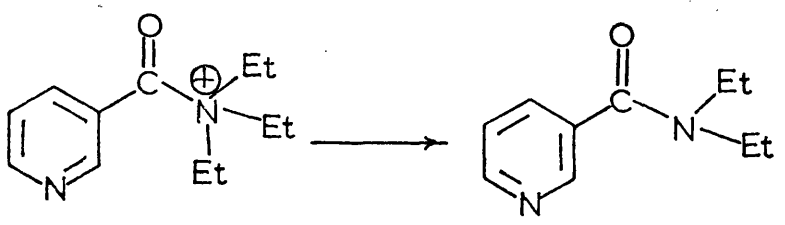


(113)



(112)

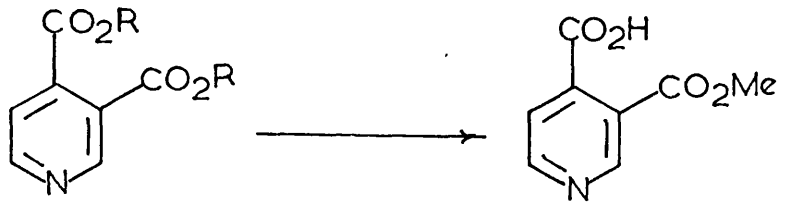
On one occasion when the acid chloride salt was reacted with an excess of triethylamine and the product reacted directly with dimethyl cadmium the secondary amide (114) was obtained. Presumably the complex (115) undergoes dealkylation in the presence of the nucleophilic reagent.



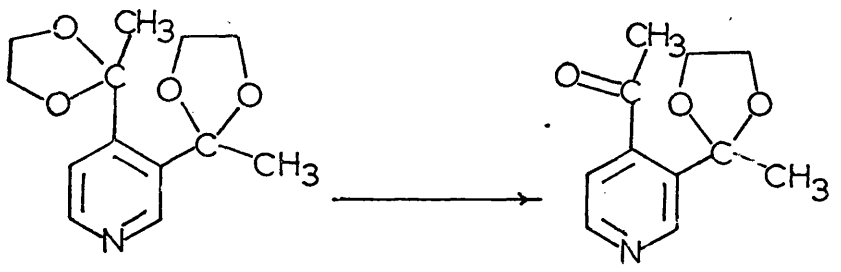
(115) (114)

(iii) From cinchomeronic acid

Our next approach to the ketone (105) depended upon the difference in reactivity between groups present at C3 and C4 of the pyridine ring. Thus, just as the diester of cinchomeronic acid (116, R = Me) may be selectively hydrolysed to give the mono ester (117)[101], we hoped to hydrolyse selectively the diketone derivative (118) to (105).

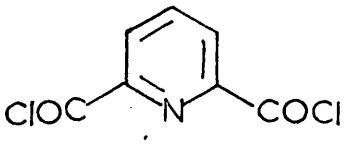


(116) (117)

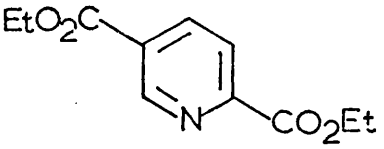


(118) (105)

The synthesis of 3,4-diacetylpyridine from cinchomeronic acid (116, R=H) seemed possible as diketo products have been prepared from the diacid chloride (119)[102], the diester (120)[103] and also various dicyanopyridines[104], although in the last case 2,3-dicyanopyridine gave (121).

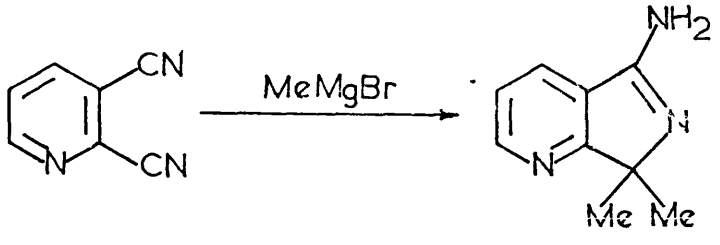


(119)

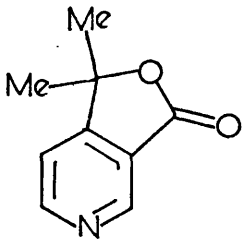


(120)

Firstly we prepared cinchomeronic acid by oxidation of isoquinoline[105] and from it synthesised samples of the corresponding diacid chloride, the dicyanide (via the diamide) and also the diethyl ester. However, reaction of these compounds with suitable reagents failed to provide the required diketone. When the first substrate was reacted with dimethyl cadmium, a very low yield of the lactone (122) was obtained. This product has been prepared previously by the action of methylmagnesium bromide upon cinchomeronic anhydride[106] and its formation is clearly analogous to (121).



(121)



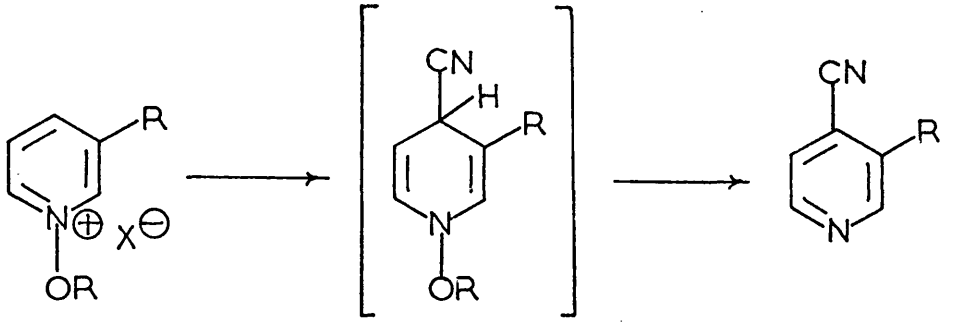
(122)

Neither the dicyanide nor the diester gave identifiable products when they were used as substrates for the preparation of 3,4-diacetylpyridine in similar reactions.

(iv) From pyridinium salts

Nucleophilic attack on pyridinium compounds may occur at either the C-2, 4 or 6 positions, however, certain nucleophiles, those capable of forming charge-transfer complexes, predominantly attack the C-4 position furnishing the corresponding 1,4-dihydropyridines[107]. The cyanide ion is included in this class and, since this function once attached to the pyridine ring system may be converted easily into an acetyl group, a relatively simple route to the desired product from the ethylene acetal of 3-acetylpyridine seemed possible.

In general, although N-alkoxy pyridinium derivatives (123) react with cyanide ion in a random manner, conditions may be chosen which favour 4-substitution[108]. In such cases the intermediate dihydro-derivative (124) is very unstable and readily loses R-OH to give the free base (125).

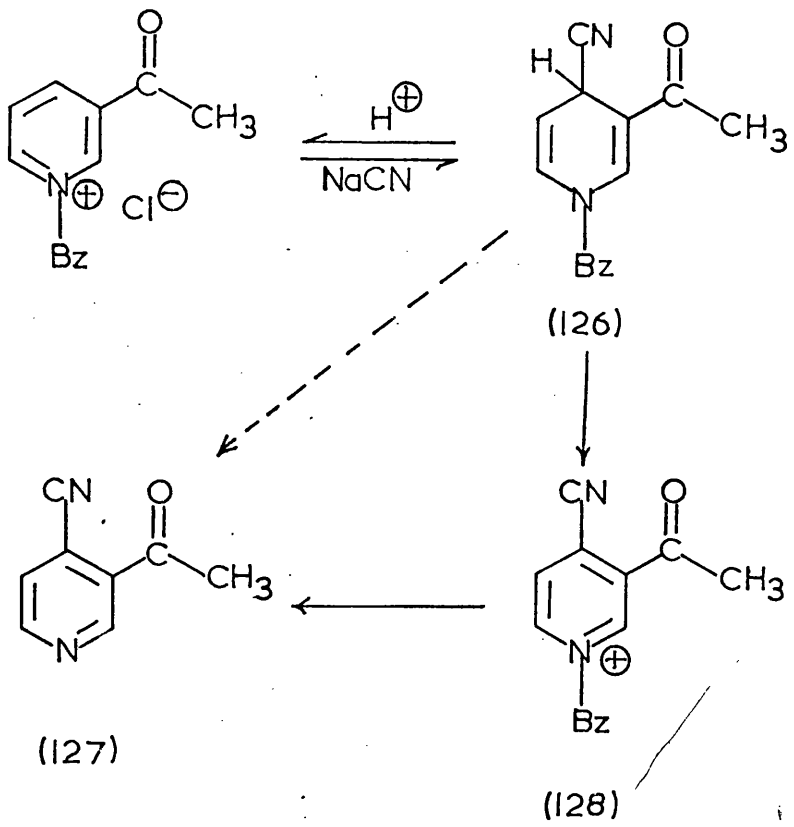


(123) (124) (125)

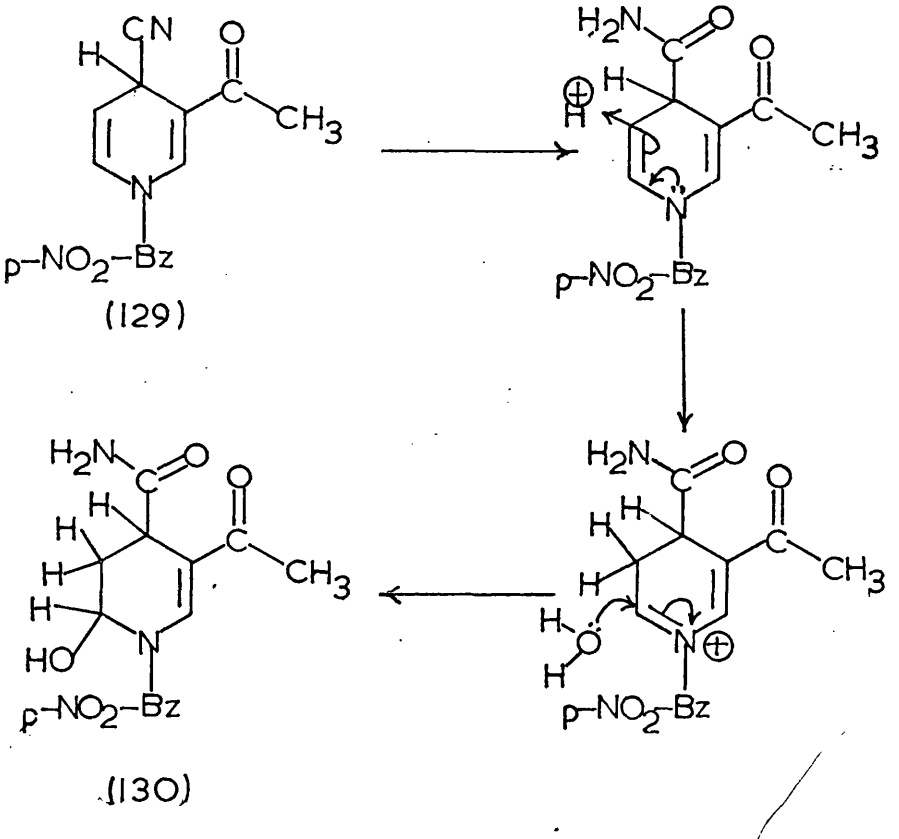
With this in mind the N-ethoxyl salt of 3-acetylpyridine was prepared and reacted with an aqueous solution of sodium cyanide. Immediately a red-brown oil separated, but attempts to purify this product were unsuccessful.

It is known that N-benzyl-3-acetylpyridinium chloride reacts cleanly with cyanide ion affording a high yield of (126)[109(a)]. Utilizing this procedure we hoped to achieve an efficient synthesis of the 4-cyanopyridine (127) (see route 13). Although (126) was obtained easily, aromatization to (127) or the N-benzyl quaternary salt (128) could not be achieved. Dehydrogenation with palladium on carbon as a catalyst in various solvents gave only complex gums and oxidation attempts with thionyl chloride or iodine were equally disappointing. Our final attempt was with dichloro-dicyano-*p*-benzoquinone, but the crude product failed to show any evidence of a cyanide group being present.

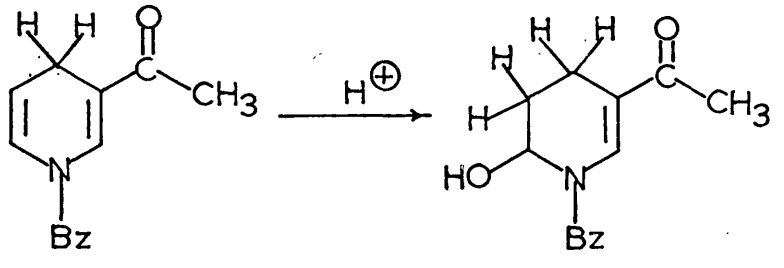
Route 13



Aromatization of the 1,4-dihydropyridine (126) to the corresponding pyridine necessitates the production of the benzyl anion or radical either by a concerted or two step process. In an attempt to expedite the aromatization step we prepared the p-nitrobenzyl analogue (129). Base treatment of this gave only tars but with dilute acid at 50° a product was obtained which, from an inspection of the infra red spectrum, contained nitro, acetyl, hydroxyl and primary amide functions. Its mass spectrum and ^{1}H-n.m.r. spectrum (Fig. 21) are interpreted in favour of structure (130) (see experimental section) and its formation may be rationalized as follows:

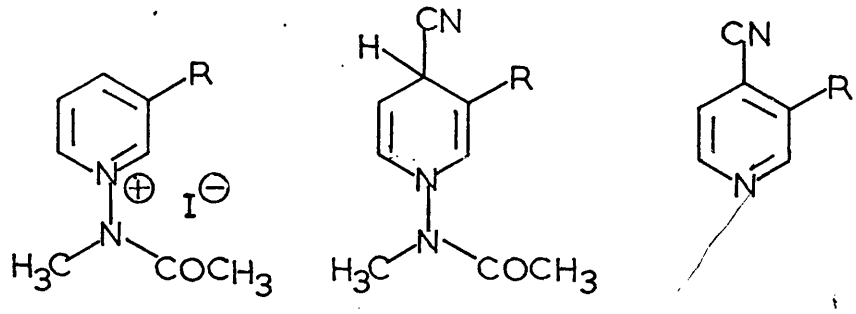


A literature search for precedents of this type of behaviour revealed that the dihydropyridine (131)[109(b)] reacts with dilute hydrochloric acid in an analogous manner to give (132).



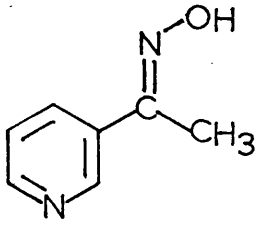
(131) (132)

At this time a paper published by a Japanese group[110] caused us to abandon our study of the benzyl compounds for an approach based on the pyridinium system (133). These workers observed that in the presence of ammonium chloride, cyanide ion added almost exclusively to the C-4 position of (133) generating the unstable 1,4-dihydro derivative (134) usually in good yield. Fragmentation of the N-N bond is easily effected giving the corresponding 4-cyanopyridine.

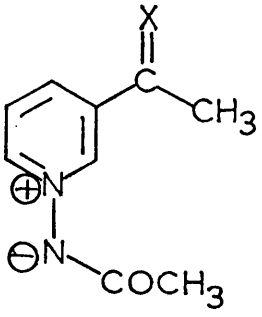


(133) (134)

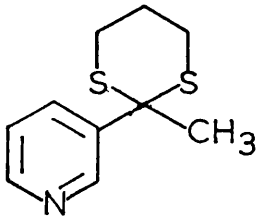
Following the same conditions we attempted to prepare the pyridinium system (133, R = $COCH_3$). However, reaction of 3-acetylpyridine with hydroxylamine-O-sulphonic acid resulted in attack at the carbonyl function since after acetylation and chromatographic separation of the product, the two oximes (135) and (136, X = N - OH) were isolated. Repetition of the experiment with the ethylene acetal (106) and the thiane derivative (137) were similarly unsuccessful as the protecting groups were lost.



(135)



(136)



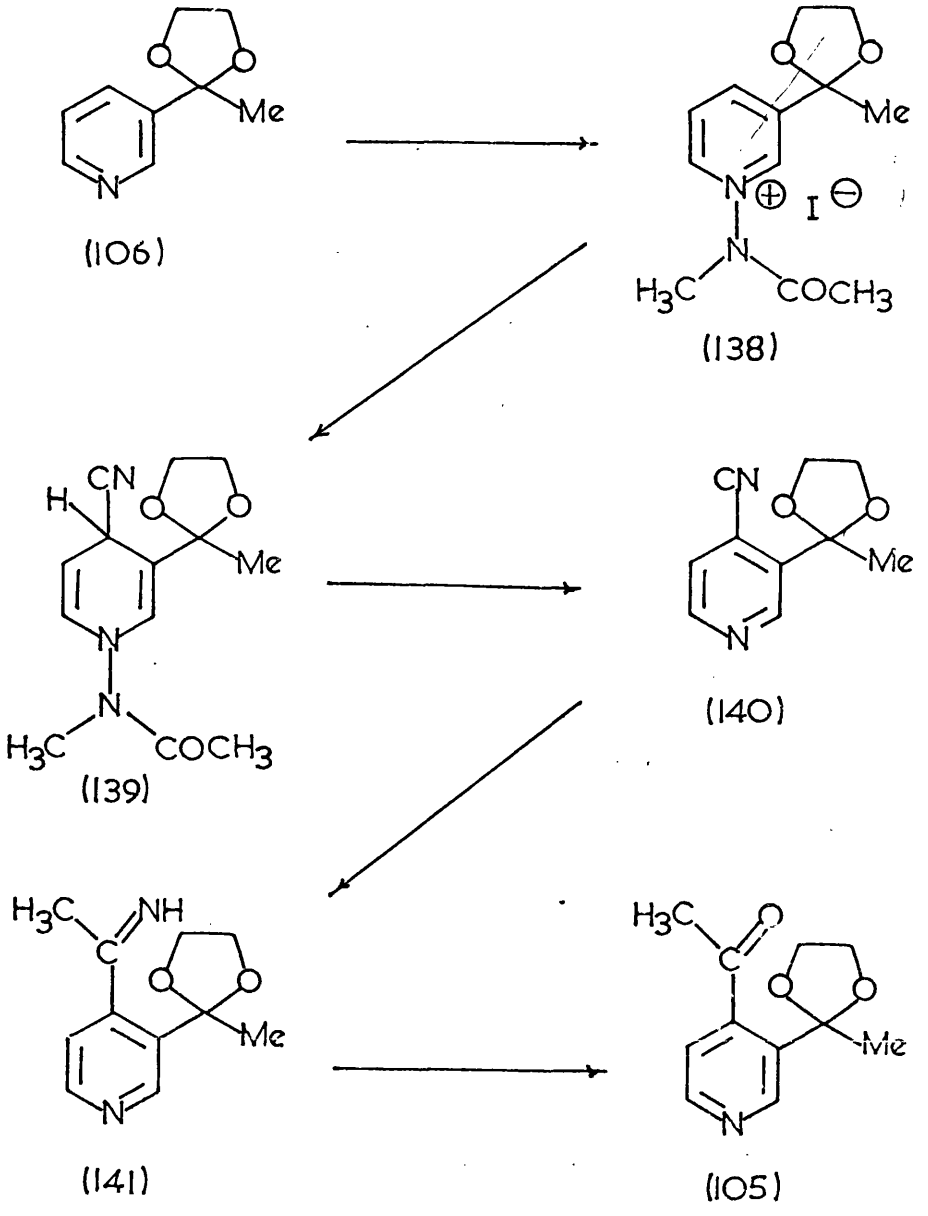
(137)

Fortunately a recent publication[111] shows that N-amination of pyridines may be achieved by using O-mesitylene sulphonyl hydroxylamine and when we treated the acetal (106) with this reagent we obtained a near quantitative yield of the N-amino salt. In order to form (138) acetylation and methylation are necessary; the first of these procedures required generation of the free base, rather than direct acetylation of the salt as stated by Suzue et al[110], but apart from this modification repetition of the published general procedure gave excellent results.

The key reaction in the projected route to 3,4-diacetylpyridine was the addition of cyanide ion to form the pyridinium intermediate (139). We were therefore very relieved when the reaction worked efficiently giving a good yield of the 4-cyano-pyridine (140). In the initial work-up procedure we were able to isolate the 1,4-dihydro--derivative (139) although our evidence for this product was confined to IR and 'H-n.m.r. data. Aromatization however, was very easy: merely passing the material down an alumina column not only caused oxidation, but served as a useful purification technique.

Success in this reaction was tinged with disappointment since treatment of the cyano-compound (140) with methyl magnesium bromide failed completely and gave a quantitative recovery of starting material. This result was the first indication of problems of steric hindrance resulting from the bulky 3-substituent. However, by employing methyl lithium the necessary conversion was achieved and (141) was obtained in high yield. To complete the synthesis (see Route 14) selective hydrolysis with hot acetic acid resulted in the formation of the ketone (105). Overall yield of (105) = 62.3%.

Route 14



Attempted condensation between (143) and (93).

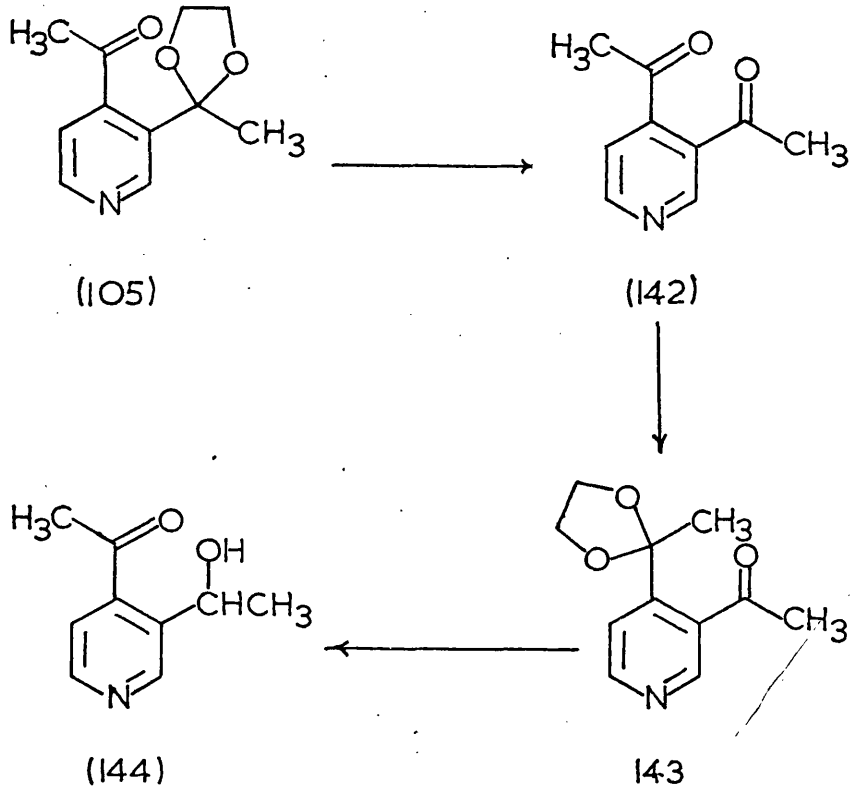
Having only a limited stock of the 5-acetamidoindoxyl derivative (93) we investigated further steps in the synthesis with the unsubstituted compound (61). Thus the usual conditions for the formation of indolinones were employed on a mixture of the ketone (105) and 1,3-diacetylindoxyl. However, after allowing to stand for seven days the reaction mixture did not contain any of the required product. Instead a large quantity of indigotin was obtained, together with much starting ketone. Attempts to effect the condensation at elevated temperatures were equally unsuccessful.

From a consideration of molecular models it seems that these failures are probably due to the steric hindrance which the rigid acetal function at C-3 imparts to the 4-acetyl group. Therefore any further attempts to utilize the acetal were abandoned in favcur of a derivative which contained a single bond joined to the α-carbon atom of the C-3 side chain. Here steric problems are much reduced.

Just as cinchomeronic acid may be selectively esterified at C-4[100] we hoped to be able to protect by acetal formation, the acetyl function of (142) at this position without affecting the acetyl function at C-3. Reduction and hydrolysis of (143) should then afford a suitable derivative (144) for condensation with the indoxyl (see route 15).

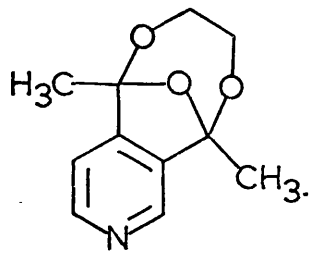
3,4-Diacetylpyridine was obtained from the acetal (105) by treatment with 20% HCl at 90°, these relatively severe conditions are further indications of the interaction of the groups at C-3

Route 15.



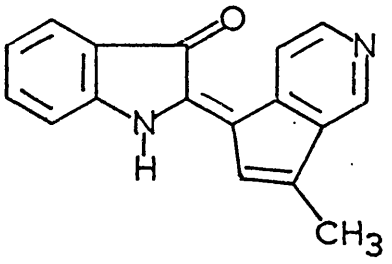
(105) (142) 143 (144)

and C-4; but when the diketone was reacted with ethylene glycol it gave a small yield of a product considered to be (145) together with a similar quantity of a mixture of (105) and (143).

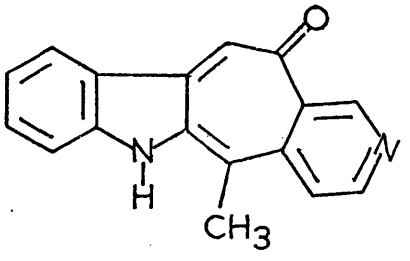


(145)

As a very speculative experiment we attempted, at this time, to combine 3,4-diacetylpyridine (142) with 1,3-diacetylindoxyl; such an interaction is likely to produce a mixture of products and after a relatively short period (3 days) the reaction was terminated. A large amount of indigotin was present in the product together with a small amount of the 3-indolinone (146).



(146)

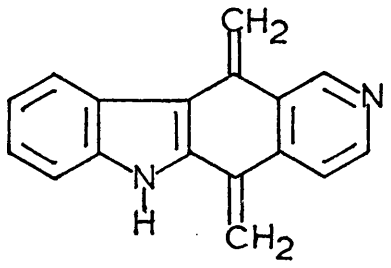


(147)

Evidence for this structure is as follows: in the mass spectrum the molecular ion is also the base peak at m/e 260. A M^+-1 ion and a peak due to the loss of 28 units (CO) are prominent features of this spectrum and bands at 3120(NH), 1630(C=C) and 1690(C=O)cm^{-1} are present in the infra red spectrum. The alternative structure (147) may be eliminated since in the 'H-n.m.r. spectrum (Fig. 22) a very low field position for the C-3 pyridyl proton reflects the anisotropic effect of the rigid indolinone system.

Cranwell and Saxton's synthesis[11] (see page 55) of ellipticine entails a condensation of indole with hexane-2,5-dione. It is conceivable that an analogous reaction might occur between indole and 3,4-diacetylpyridine to give (148) which on reduction should afford ellipticine, although if anything, it is more probable that

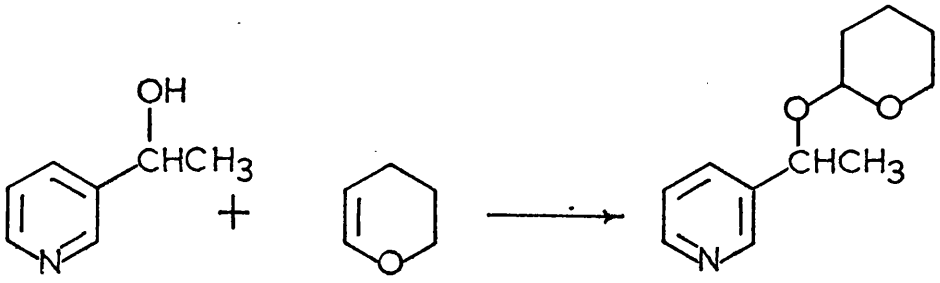
isoellipticine would be the major product. In practice, however,



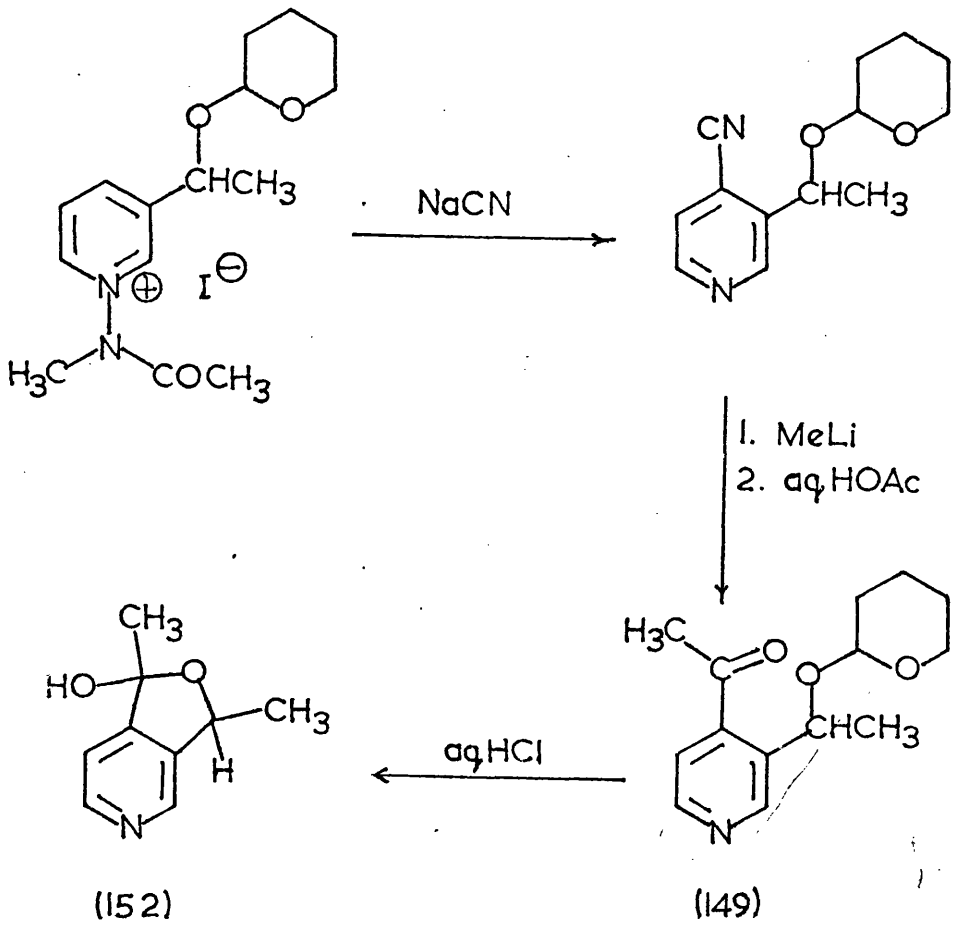
(148)

the reaction gave no identifiable products.

With the possibilities of using 3,4-diacetylpyridine apparently exhausted we now moved our attention to the use of the protected alcohol (149). Thus 2,3-dihydropyran was condensed with the hydrochloride salt of (150), dissolved in dry dimethylformamide to give (151). With this compound we followed exactly the same route by which the ketone (105) was prepared except that the final imine hydrolysis with acetic acid was effected at room temperature (overall yield 65%). Under more severe conditions of hydrolysis the protecting group is cleaved and the product isolated is then the hemiacetal (152).

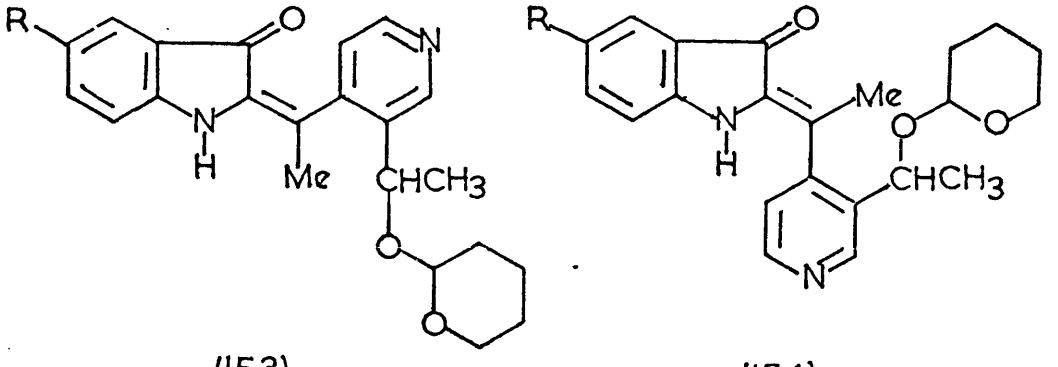


(150) (151)



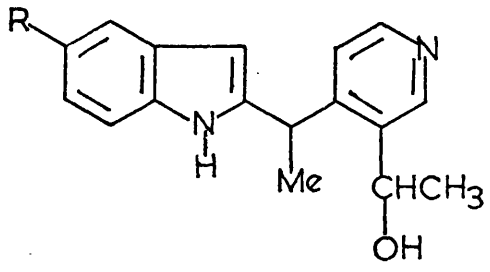
(152) (149)

Under strongly basic conditions, reaction of (149) with 1,3-diacetylindoxyl proceeded smoothly and the isomeric indolinones (153 and 154 R=H) crystallized from the reaction mixture (yield 74.5%).



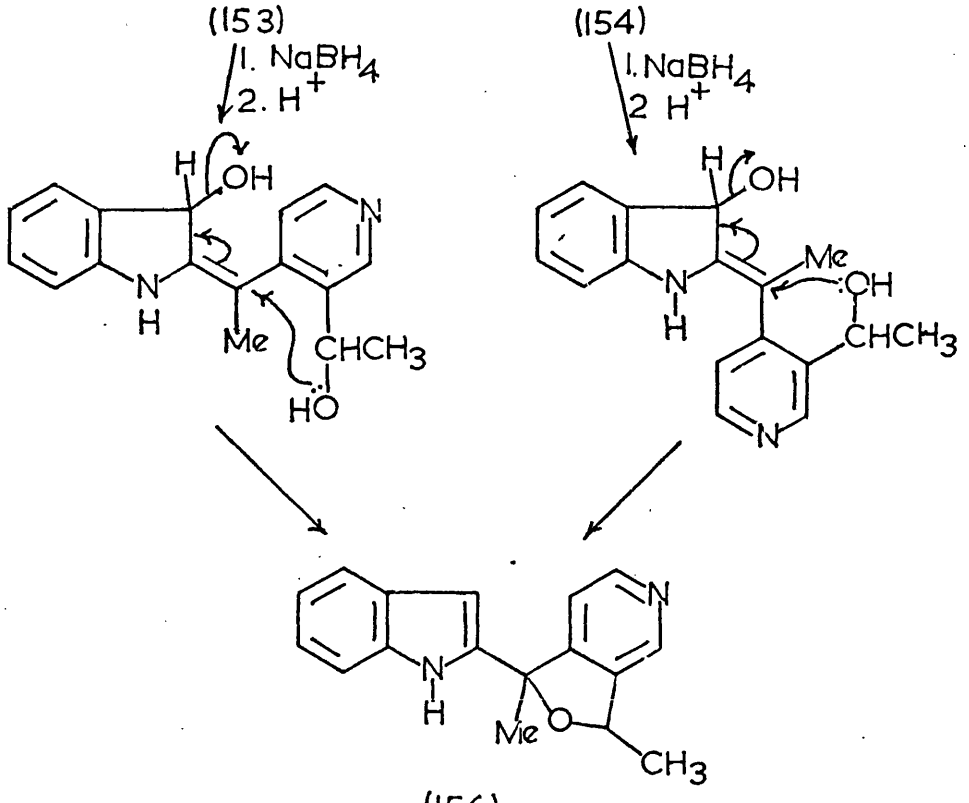
(153) (154)

As the next step sodium borohydride reduction and acid catalysed dehydration gave the alcohol (155, R=H).



(155)

An initial small scale reaction provided further evidence that a 1,2 addition (see page 94) was the first stage of the reduction. Presumably insufficient reducing agent was employed as the only product isolated was the indole (156).

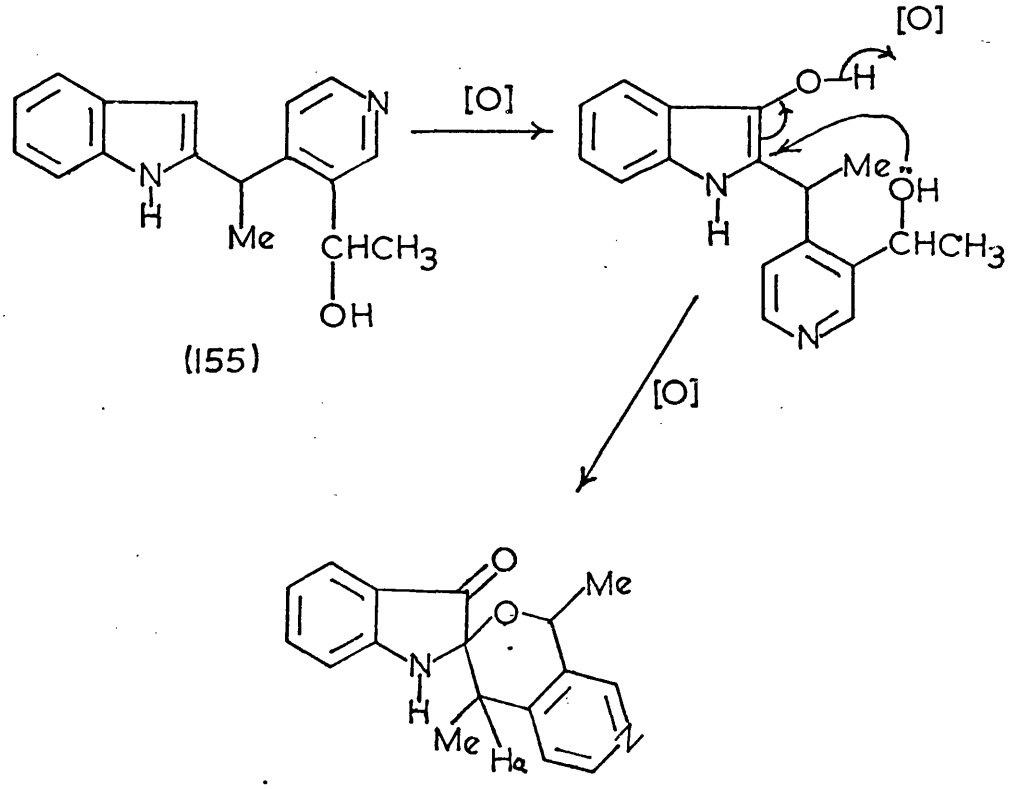


(156)

We were surprised to find that ring closure of the alcohol (155) did not occur easily. Only acid conditions comparable to those required for the methoxy analogue were successful and of the other techniques tried, treatment with phosphoryl chloride in pyridine solution offered the best alternative, giving dihydroellipticine. On the whole, however, this approach was unsatisfactory because of tar formation.

Oxidation of the pyridyl side chain to an acetyl group should be possible and ring closure to the ellipticine system should then be spontaneous. A number of reagents were investigated with this objective in mind.

Potassium dichromate in acetic acid attacked the indole nucleus and gave the spiro-compound (157), probably by the mechanism indicated below:



(155)

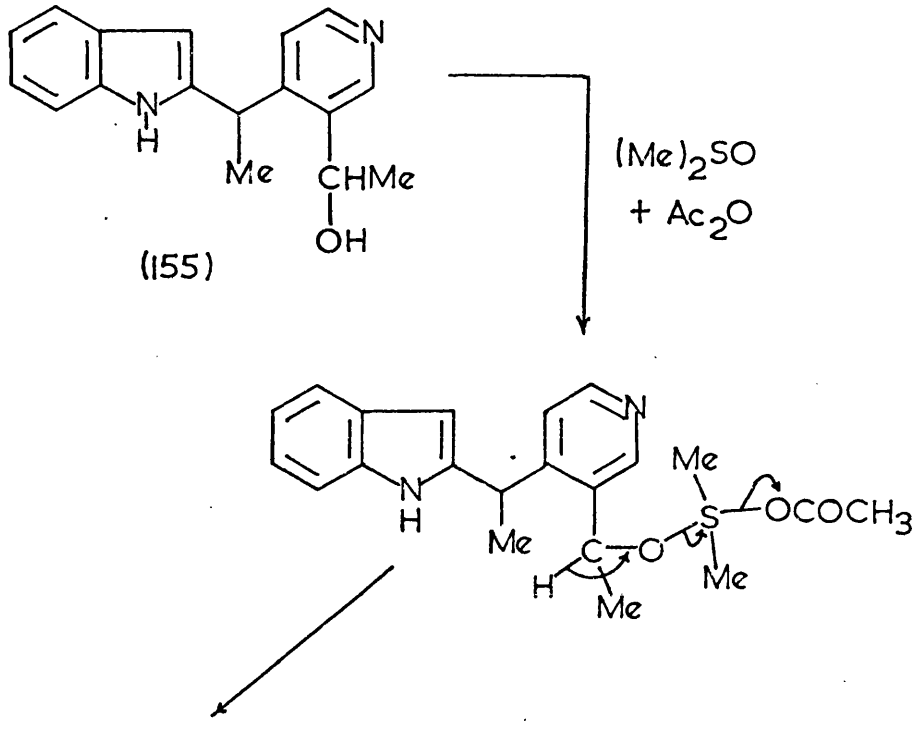
(157)

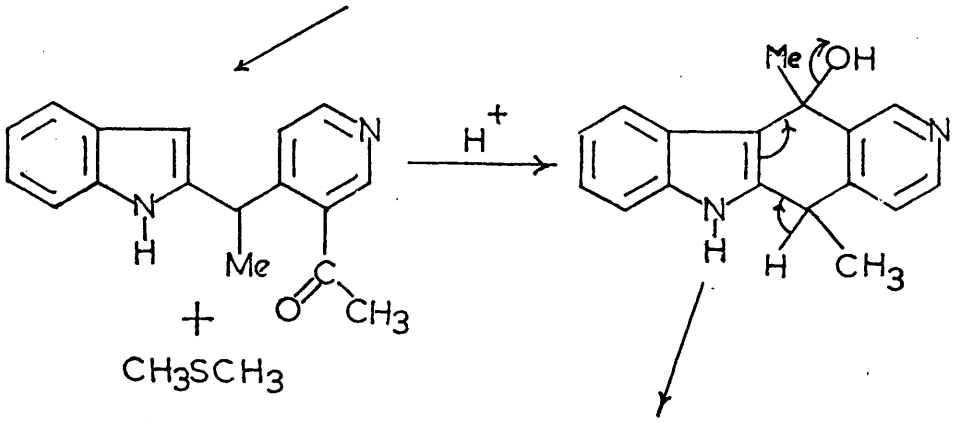
The structural allocation (157) is consistent with the following physical data: the mass spectrum exhibits a molecular ion (base peak) at m/e 280 with loss of 28 units (CO) as the major fragmentation. In the infrared spectrum bands are present at 3140(NH), 1710(C=O) and 1060cm^{-1} (C=O). The proton (a) gives rise to a quartet at 3.35p.p.m. in the ^{1}H n.m.r. spectrum (Fig. 23) and the C_3-H indolyl proton resonance, normally at about 6.3 p.p.m. (in CD_3SOCD_3), is absent.

Decreasing the severity of the oxidising conditions, however, gave better results, thus treatment of (155, R=H) with manganese dioxide furnished a low yield of ellipticine, but with a mixture of dimethyl sulphoxide and acetic anhydride a yield of 65% was achieved.

Clearly the indole ring of derivatives of the type (155) is sensitive and only very mild oxidising agents may be employed to oxidise the alcoholic function of the pyridyl side chain. Dimethylsulphoxide and acetic anhydride probably react with this function in the manner shown in Route 16.

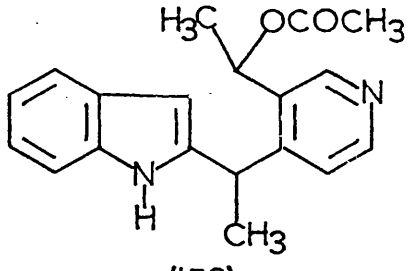
Route 16.





ELLIPTICINE

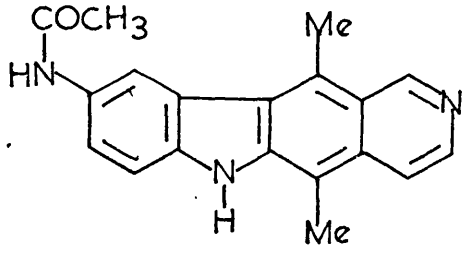
At present we are unable to comment meanfully upon the precise order of events leading ultimately to ellipticines, but it appears however, that the major by-product in the reaction sequence is the O-acetate (158) so that an excess of acetic anhydride in the reaction is undesirable.



(158)

With the optimum conditions for the oxidative cyclisation established, we attempted to synthesize 9-acetamidoellipticine (159). Reaction of (149) with the acetamidoindole (93) furnished the indolinones (153 and 145, R = $NHCOCH_3$). However, unlike the unsubstituted analogues these compounds did not crystallize from the reaction mixture and it was necessary to use solvent extraction to recover the product. From an analysis of spectral data of the crude material we suspected deacetylation had occurred and concluded that this was

the reason why solvent extraction was necessary. However, after reacetylation of the crude condensation product the indolinones (153 and 154, R=$NHCOCH_3$) were obtained and the remaining steps in the syntheses were implemented without problems. 9-Acetamidoellipticine (159) was obtained as yellow needles m.p. 245-50 (dec), from ethanol in 49% yield from the parent indolinones.



(159)

By employing this synthesis a large quantity of this compound is currently being prepared with the intention of converting it into a number of derivatives to study structure-activity relationships of the pyrido[4,3b]carbazole system.

Experimental

Attempted formation of N-(2-carboxy-6-nitrophenyl)glycine* or diester

(a) Basified (NaOH) solutions of 3-nitroanthranilic (2.3g) and chloroacetic acid (1 mole equiv.) were mixed (total volume 35ml) and maintained at 50° for 24h. On cooling and acidifying 3-nitroanthranilic acid precipitated (98% yield).

(b) A similar mixture to the above was heated under reflux for 12h. in the presence of NaI (0.4g) and Cu powder (0.5g) but again only starting material was recovered.

(c) To a solution of ethyl-3-nitroanthranilate (50mg) in dry DMF (2ml) containing sodium hydride was added ethyl bromoacetate (1 mole equiv.) and the mixture stirred and heated to 80° for 5h. Evaporation of the mixture *in vacuo* followed by ether-water extraction furnished unchanged ethyl-3-nitroanthranilate.

* Analogous reactions performed on N-(2-carboxy-3-nitrophenyl)glycine gave similar results.

3-(1-Chloroethyl)pyridine. - 1-(3-Pyridyl)ethanol (20g) (from a sodium borohydride reduction of 3-acetylpyridine) was dissolved in dry benzene (50ml) and thionyl chloride (20ml) was added dropwise, maintaining the temperature at 5-10°. The mixture was evaporated under reduced pressure to give a brown gum, which was taken up in cold water and washed with ether. The aqueous solution was made basic with solid sodium hydrogen carbonate and extracted with ether. The ethereal solution was washed, dried ($MgSO_4$), and evaporated under reduced pressure to yield the chlorinated compound as a mobile unstable brown liquid (22g, 96%), ν_{max}, 650cm^{-1}.

3-(1-Methoxyethyl)pyridine (60)[77]. - 3-(1-Chloroethyl)pyridine (22g) was added to dry methanol (150ml) containing sodium (5g). The mixture was heated under reflux for 5h. The precipitated NaCl was removed by filtration and the methanol was evaporated under reduced pressure to yield a dark brown mobile liquid. This was distilled to give 3-(1-methoxyethyl)pyridine (16.5g, 78%), b.p. 57° at 4 mmHg, m/e 137 and 106 (P), ν_{max} 1100 (OMe) and 2800cm^{-1}.

1,4-Diacetyl-1,4-dihydro-3-(1-methoxyethyl)pyridine (70)[77]

3-(1-Methoxyethyl)-pyridine (17.6g) was dissolved in acetic anhydride (300ml) and the temperature of the stirred solution reduced to -2°. Zinc dust (208g) was added portionwise to the solution (4h.) maintaining the temperature at -2° during the addition and then for a further 8h. The mixture was stirred at RT for 2 days. Removal of the zinc and zinc acetate by filtration followed by evaporation of the acetic anhydride at 40° *in vacuo* yielded a viscous yellow oil. Heating a solution of this in

methanol for 5h gave as the non-basic product 1,4-diacetyl-1,4-dihydro-3-(1-methoxyethyl)pyridine (70) Yield 34%, ν_{max} 1710(CH_3CO), 1670(CH_3CON), 1630(C=C)cm^{-1}.

Conversion of (70) to 4-acetyl-3-(1-methoxyethyl)pyridine(59)

(a) With chromium trioxide

To a stirred solution of (70) (1g) in glacial acetic acid was added an aqueous solution of chromium trioxide (200mg) over a period of $\frac{1}{4}$h. After a further $\frac{3}{4}$h stirring, excess isopropyl alcohol was added and then the mixture evaporated in vacuo. The residual gum was worked up for bases giving (59), (yield 34%) as yellow oil.
M^+ 179, 164(P), ν_{max} 1700($COCH_3$)cm^{-1}.

(b) With nitrous acid

A solution of (70) (200mg) in chloroform (10ml) and dilute aqueous nitrous acid solution (10ml) were mixed and stirred vigorously overnight at R.T. The aqueous layer was separated basified and extracted with chloroform but, after drying ($MgSO_4$) and evaporation, this gave only a slight residue of an unidentifiable product. The non-basic layer consisted only of resinous material.

(c) With iodine

To the 1,4-diacetylpyridine derivative (70) (700mg) dissolved hot benzene (50ml) was added a solution of iodine (1.53g) in benzene (100ml) over 30min. After a further 30min., a deep red-brown gum separated, therefore the liquid phase was decanted, this was washed with sodium sulphite solution and worked up for bases but gave only a trace of a gum which was not examined further.

(d) With quinones

A mixture of (70) (200mg), p-benzoquinone (310mg) and xylene (12ml) was heated to reflux overnight. After allowing to cool, the solution was extracted with 2N.HCl and the aqueous layer washed with ether, basified and re-extracted with chloroform, providing (59) in 59% yield. A similar yield (58%) was obtained by employing chloranil.

Repeating the above procedure on a large scale (40g of (70)), however, gave only a 5% yield of (59), most of the product remaining in the xylene layer and consisting of an intractible tar. The etherial washings were collected and reduced in bulk causing crystallization of hydroquinone. Evaporation of the filtrate furnished a red oil and when distilled (112° at 0.8mm) this provided a viscous orange coloured oil which slowly crystallized. Trituration with $CHCl_3$ then gave more hydroquinone but on evaporation of the filtrate the quinol acetate (73) was obtained m.p. $59-61^{\circ}$ (petrol $60-80^{\circ}$)(lit.[112] $62-3^{\circ}$).

(e) With triethoxytetrafluoroborate (Meerwein's reagent)

Meerwein's reagent (1 mole equ.) was added over 2 minutes to a stirred solution of (70) (510mg) in $CHCl_3$ (12ml.). After a further 30 min. the solvent was evaporated and saturated $NaHCO_3$ solution (25ml) added to the residue. By extraction with $CHCl_3$ and working up for bases a small quantity of an orange oil, M^+ 149 (possibly (75)) was obtained.

(f) By heating in Methanolic solution

The dihydro-pyridine (70) (10g) was heated for 4 days in methanol (11). The solvent was then removed and the residual oil was worked up for basic and non-basic components, giving 4-acetyl-3-methoxyethylpyridine (29%)

Repeating the procedure in the presence of a catalytic amount of dibenzoyl peroxide, or ferric chloride, increased the yield of (59) to 35-40%.

3-Ethoxy-5-nitroindole (77)

The nitroindole (69) (4.2g) in ethanol (50ml) was heated under reflux with aqueous 20% sulphuric acid (10ml) under nitrogen for 1h. On dilution and extraction with ether, compound (4) was obtained as a gum which slowly crystallized and was recrystallized from aqueous ethanol to give pale yellow prisms (30g) m.p. 112°, m/e 206, 178(base), 132, and 131 ν_{max} 3400 (NH), 1620 (C=C), 1510 (N=O), and 1330cm^{-1} (NO), λ_{max} 268sh (ε 7250), 288 (13,000), and 337 (5230)nm, δ($CDCl_3$) 0.95 (3H, t, J 7 Hz, $CH_2.\underline{CH_3}$), 3.65 (2H, q, J 7 Hz, $\underline{CH_2}.CH_3$) 6.6 (1H, s, 2-H), 7.0 (1H, d, J 9 Hz, 7-H), 7.55 (1H, 2 x d, J 9 and 2.5 Hz, 6-H), and 8.05 (1H, d, J 2.5 Hz, 4-H) (Found: C, 58.0; H, 5.0; N, 13.5. $C_{10}H_{10}N_2O_3$ requires C, 58.25; H, 4.9; N, 13.6%).

(E)- and (Z)-2-{1-[3-(1-methoxyethyl)-4-pyridyl] ethylidene}-5-nitroindolin-3-one (78) and (79).

(a) A mixture of 3-ethoxy-5-nitroindole (4.8g) and 4-acetyl-3-(1-methoxyethyl)pyridine (4.3g) in aqueous 10% hydrogen bromide (125ml) was refluxed for 10 min. under nitrogen atmosphere and then kept at room temperature for 72h. Basification and extraction with

chloroform furnished a red oil together with a considerable amount of a deep purple insoluble solid which could not be purified. Trituration of the oil with ethanol afforded the E-isomer (78), m.p. 250° (decomp.) (from ethanol), and evaporation of the ethanolic filtrate gave the Z-isomer (79), m.p. 225-230°; total yield 4%; m/e 339, 307 (base), 292, and 280.

The (E)-isomer showed ν_{max} 1688 (CO), 1630 (C=C), 1505, and 1325cm^{-1}, λ_{max} 256 (ε 12,500), 266 (12,900), 287sh (14,000), 297 (15,100), 372.(10,100), 420sh (5900), and 444 (5150) nm, δ($^{2}H_{5}$ pyridine) 30° (Fig. 13). 1.45 and 1.51 (3H, 2 x d, J 6 Hz, CH.CH$_3$), 2.32 and 2.38 (3H, 2 x s, C.CH$_3$), 3.19 and 3.28 (3H, 2 x s, OMe), ca 4.6 (1H, 2 x interleaving q, CH.CH$_3$), 7.02 (1H, d, J 8.5 Hz, 7-H), 7.20 (1H, d, J 5 Hz, 5'-H), 8.32 (1H, 2 x d, J 8.5 and 2.0 Hz, 6-H), 8.56 (1H, d, J 2.0 Hz, 4-H) 8.6 (1H, d, J 5 Hz, 6'-H), and 9.12 (1H, s, 2'-H). From the temperature dependence of this spectrum, Ea, the potential energy barrier to rotation, is calculated to be 71 kJ mol^{-1}.

The (Z)-isomer showed ν_{max} 1695 (CO), 1635 (C=C), 1605, 1515, and 1325 cm^{-1} (the electronic spectra of E and Z-isomers are identical,) δ[$(CD_3)_2SO$] (Fig. 12) 30° 1.35 (3H, d, J 6Hz, CHCH$_3$), 2.6 (3H, s, C.CH$_3$), 3.3 (3H, s, OMe), ca. 4.3 (1H, q, CH.CH$_3$), 7.0 (1H, d, J 9 Hz, 7-H), 7.3 (1H, d, J 5 Hz, 5'-H), 8.2 (1H, 2 x d, J 9 and 2 Hz, 6-H), 8.25 (1H, d, J 2 Hz, 4-H), 8.6 (1H, d, J 5 Hz, 6'-H), 8.7 (1H, s, 2'-H), and 9.7 (1H, s, NH). In the case of this isomer the n.m.r. spectrum obtained was that of the enantiomeric mixture, the

coalescence temperature being below 30° (Found: C, 63.5; H, 5.0; N, 12.5; $C_{18}H_{17}N_3O_4$ requires C, 63.7; H, 5.05; N, 12.4%).

(b) The indolinone (62) (2g) in concentrated sulphuric acid (6ml) at 0° was treated dropwise with potassium nitrate (1.2 mol. equiv.) in concentrated sulphuric acid during 10 min. The mixture was then poured on ice (30g) basified with sodium hydrogen carbonate and extracted with chloroform to give, after removal of solvent, a deep red gum which was worked up to give a mixture of (78) and (79) (750mg).

(E) and (Z)-2-{1-[3-(1-methoxyethyl)-4-pyridyl]ethylidine} indolin-3-one (62) and (63) [77]

4-Acetyl-3-(1-methoxyethyl)pyridine (6g) in 50% aqueous methanol (80ml) containing potassium hydroxide (16g) was added to the acetate (61) (7.27g) in a nitrogen-purged flask. The vessel was tightly stoppered and left 5 days at room temperature. Filtration under nitrogen then afforded an isomeric mixture of (62) and (63), m.p. 180-1° (lit.[77], 180-1°) and 181-2° (lit.[77], 181-2°) respectively. Total yield 86.6%.

Sodium borohydride treatment and subsequent dehydration of the indolinones (62) and (63).

(a) (incomplete reaction)

The indolinone mixture (62) and (63) (2.2g) was dissolved in boiling 95% ethanol (100ml) and reduced portionwise with sodium borohydride. After 20 minutes on the steam-bath the ethanol was evaporated and the residue partitioned between chloroform and water. Evaporation

of the dried organic phase furnished a blue sticky solid. This was taken up in methanol (80ml) saturated with hydrogen chloride and then the solvent evaporated. Partitioning the residue between chloroform and sodium carbonate solution furnished a gummy organic extract which by trituration with ethanol crystallized to give (64), m.p. 164-5° (lit.[77], 165-6°). Partial evaporation of the filtrate provided a further quantity of (64), but upon washing the final residue with acetone, filtering, and adding 2-3 drops of H_2O the vinyl indole (81, R=H) crystallised m.p. 176-7° (aq EtOH) m/e 278, 363, 346 and 231 (Base), ν_{max} 3170(NH), 1620(C=C) and 1120(C-O)cm^{-1}, λ_{max} 293sh(6950), 306(8200) and 312sh(8100)nm., δ ($CDCl_3$)(Fig. 15) 1.36(3H, d, J 6 Hz, $\underline{CH_3}CH$), 3.10 (3H, s, OMe), 4.48 (1H, q, J 6Hz, $CH_3\underline{CH}$), 5.13 and 5.80 (2H, 2 x s, $C=\underline{CH_2}$), 6.16 (1H, d, J 2Hz, C_3-H), 6.95-7.60 (5H, complex, benzenoid and C_3'-H) 8.45-8.70(2H, complex C_6'-H and NH) and 8.22 (1H, s, C_2'-H) (Found: C, 77.6; H, 6.5; N, 10.0. $C_{18}H_{18}N_2O$ requires C, 77.7; H, 6.5; N, 10.1%).

(b) Repeating the above procedure, but this time following the reaction course by observing changes in the UV spectra, gave (64) in 90% yield.

2-[1-(3-Vinyl-4-pyridyl)ethyl]indole (85). The indole (64) (200mg) in concentrated sulphuric acid (10ml) was heated for 30 min. at 100°, cooled, and poured on ice. Basification and extraction with ether yielded (85) as prisms (152 mg, 86%), m.p. 140-141°, m/e 248 and 233 (base), ν_{max} 3110, 1625, 1610, and 1540cm^{-1}, λ_{max} 260 (ε 11,900), 280sh (10,500), and 293 (8,100)nm,

δ ($CDCl_3$) (Fig. 24), 1.6 (3H, d, J 7 Hz, CH.$\underline{CH_3}$), 4.4 (1H, q, J 7 Hz, $\underline{CH}$.CH_3), 5.25, 5.4, and 5.7 (2H, 3 x d, J 1.5 Hz, CH=$\underline{CH_2}$), 6.4br (1H, s, 3-$\underline{H}$), 6.7-7.2 (5H, m, 4-, 5-, 6-, 5'-$\underline{H}$, and $\underline{CH}$=CH_2), 7.5 (1H, m, 7-$\underline{H}$), 8.25 (1H, d, J 5 Hz, 6'-$\underline{H}$), 8.5 (1H, s, 2'-$\underline{H}$), and 8.7br (1H, s, $\underline{NH}$) (Found: C, 82.4; H, 6.7; N, 11.3. $C_{17}H_{16}N_2$ requires C, 82.2; H, 6.7; N, 11.3%).

2-{1-[3-(1-Methoxyethyl)-4-pyridyl]ethyl}-3,5,7-trinitroindole (86)

The indole (64) (1.2g) in concentrated sulphuric acid (10ml)* was treated dropwise with a solution of potassium nitrate (4 mole equiv.) in sulphuric acid at 0°. After the addition (ca. 30min), the mixture was poured on ice, basified and extracted with ethyl acetate. Evaporation of the extract and trituration of the residue with ether afforded a yellow solid which crystallized from ethanol as prisms (1.19g) m.p. 235° (decomp.) m/e 415, 368, 337 (base), and 321, ν_{max} ca. 3400 (EtOH, NH), 1600, 1540, and 1105 cm^{-1}, λ_{max} 272 (ε 17,800), 330(11,500), and 411(10,200) nm δ[$(CD_3)_2SO$] (Fig. 16) 1.0 (3H, t, J 7 Hz, $\underline{CH_3}$.CH_2.OH), 1.4 (3H, d, J 6.5 Hz, $\underline{CH_3}$.CH(OMe), 1.75 (3H, d, J 6.5 Hz, $\underline{CH_3}$C), 3.1 (3H, s, O$\underline{Me}$), 3.5 (2H, q, J 7 Hz, CH_3.$\underline{CH_2}$.OH), 4.8 (1H, q, J 6.5 Hz, $\underline{CH}$(OMe)Me), 5.5 (1H, q, J 6 Hz, $\underline{CH}$.CH_3), 7.0 (1H, d, J 5 Hz, 5'-$\underline{H}$), 8.35 (1H, s, 4-$\underline{H}$), 8.5 (1H, d, J 5 Hz, 6'-$\underline{H}$), and 8.7 (2H, s, 6- and 2'-$\underline{H}$) (Found: C, 52.3; H, 4.9; N, 15.1. $C_{18}H_{17}N_5O_7$,C_2H_5OH requires C, 52.1; H, 5.0; N, 15.2%).

* The n.m.r. spectrum of the indole (64) in sulphuric acid exhibits a broad two-proton singlet at δ 4.0. This is absent in the spectrum of a solution in deuteriochloroform, where the C-3 proton gives a clearly defined singlet at δ 6.4.

2-{1-[3-(1-Methoxyethyl)-4-pyridyl]ethyl}-3,5,dinitroindole (87)

The foregoing reaction was repeated with 1 mol. equiv. of potassium nitrate. After purification, the product (87) was obtained as yellow prisms from acetone, (486mg), m.p. 140° (decomp), m/e 370 (weak), 323, and 292 (base), ν_{max} 1600, 1540, 1520, 1350 and 1105 cm^{-1}, λ_{max} 255 (ε 22,300), 320br (10,600), and 412(6860) nm, δ($CDCl_3$) (Fig. 17) 1.45 (3H, d, J 7 Hz, CH(OMe).CH_3), 1.74 and 1.70 (3H, 2 x d, J 7 Hz, CH.CH_3) (diastereoisomerism; see ref. (77)), 3.1 and 3.2 (3H, 2 x s, OMe), 4.85br (1H, q, J 7 Hz CH(OMe).CH_3), 5.5br (1H, q, J 7 Hz, CH.CH_3), 7.05 (1H, m, 5'-H), 7.8 (1H, d, J 9 Hz, 7-H), 8.25 (1H, 2 x d, J 9 and 2 Hz, 6-H), 8.45br (1H, d, J 5 Hz, 6'-H), 8.6 (1H, s, 2'-H), and 8.9 (1H, d, J 2 Hz, 4-H) (Found: C, 58.2; H, 4.9; N, 15.1. $C_{18}H_{18}N_4O_5$ requires C, 58.4; H, 4.9; N, 15.1%).

2-{1-[3-(1-Methoxyethyl)-4-pyridyl]ethyl}-3-nitro-5-aminoindole (88).

A solution of (87) (100mg) in ethanol was hydrogenated at 70 p.s.i. over Adam's catalyst during 4h. Removal of the catalyst and solvent furnished a gum which solidified on trituration with ether. Crystallization from aqueous ethanol gave (88) as small yellow prisms (yield 21%), m.p. 258-60 (dec.), m/e 340 (M^+), 276, 262 (Base) and 248, ν_{max} ca. 3410 and 3320 (NH_2), 1630 (N=C), 1590cm^{-1}, λ_{max} (ε) 224 (2450), 258 (1220), 265sh (1130), 305(5670), 365(7700), δ[$(CD_3)_2SO$] (Fig. 25) 1.4 (3H, d, J 7 Hz, CH(OMe)CH_3), 1.65 and 1.70 (3H, 2 x d J 7 Hz CH_3C), 3.05 and 3.15 (3H, 2 x s, OMe), 4.8 br (1H, q, J 7 Hz . CH(OMe)CH_3), 5.35 br (1H, q, J 7 Hz, $CHCH_3$), 6.65 br(1H, d, 8 Hz, 6 - H), 6.9 (1H, 2 x d, J 6 Hz, 5'-H),

7.24 (1H, d, J 8 Hz, 7-H), 7.28 (1H, s, 4-H), 8.35 (1H, d, 6'-H) and 8.55 (1H, s, 2'-H). (Found: C, 63.4; H, 5.9; N, 16.4. $C_{18}H_{20}N_4O_3$ requires C, 63.5; H, 5.9; N, 16.5%).

5-Amino-2-{1-[3-(1-methoxyethyl)-4-pyridyl]ethyl}indole (89)

Reduction of the indolinones (78) and (79) with sodium borohydride in boiling ethanol followed by acid treatment gave, on work-up, a small amount of an almost colourless solid m/e 295, 248 (base), and 233 , which on acetylation afforded (101) (see later).

2-{1-[3-(1-Methoxyethyl)-4-pyridyl]ethylidene}-5-nitro-indolin-3-ol (90).

The indolinones (78) and (79) (500mg) in 95% ethanol (50ml) were treated with sodium borohydride in portions at room temperature. After 15 min. the solvent was evaporated off and the residue partitioned between chloroform and water. Concentration of the chloroform layer afforded a red gum which when triturated with ether gave an orange solid. This when recrystallized from ethanol provided deep red crystals (76mg), m.p. 194-195° (subsequent crops from the mother liquor were yellow and melted in the range 145-155°), m/e 341 (v.weak), 323, 307, 292 (base), and 278, ν_{max} ca. 3320, 1620, 1510, 1320 and 1180cm^{-1}, λ_{max} 254 (ε 8950) and 410 (15,400) nm, δ [$(CD_3)_2SO$] 1.40 (3H, d, J 6 Hz, CH.CH$_3$), 2.0 (3H, s, CH$_3$), 3.2 (3H, s, OMe), 4.5 (1H, q, J 6 Hz, CH.CH$_3$), 5.6 (1H, s, C(OH)H), 6.8 (1H, d, J 7 Hz, 5'-H), 7.25 (1H, d, J 8 Hz, 7-H), 8.0 (1H, d, J 2 Hz, 4-H), 8.15 (1H, 2 x d, J 7 and 2 Hz, 6-H), 8.45 (1H, d, J 7 Hz, 6'-H), 8.6 (1H, s, 2'-H), and 9.75 (1H, s, NH) (an additional peak, probably due to OH plus water is observed at δ 3.1). This material could not be dried (see later) and consistent analytical figures were not obtained.

2-{1-[3-(1-Methoxyethyl)-4-pyridyl]vinyl}-5-nitroindole (91)

A solution of the alcohol (90) (50mg) in dry benzene was heated in a Dean-Stark apparatus for 12h. Removal of the solvent and crystallization of the residue from aqueous ethanol gave (91) as yellow prisms (22mg, 46.5%), m.p. 239°, m/e 323, 291 (base), and 276, ν_{max} 1620sh, 1610, 1592, 1515, 1330 and 1110 cm^{-1}, λ_{max} 288sh (ε 36,800), 293(37,800), 310sh (13,070), and 341(9950) nm., δ ($CDCl_3$) (Fig. 26), 1.3 (3H, d, J 6 Hz, CH.CH_3), 3.0 (3H, s, OMe), 4.35 (1H, q, J 6 Hz, CH.CH_3), 5.3 and 6.2 (2H, 2 x s, C=CH_2), 6.21 (1H, s, 3-H), 7.25 (1H, d, J 5 Hz, 5'-H), 7.5 (1H, d, J 9 Hz, 7-H), 8.0 (1H, 2 x d, J 9 and 2 Hz, 6-H), 8.45 (1H, d, J 2 Hz, 4-H), 8.55 (1H, d, J 5 Hz, 6'-H), and 8.7 (1H, s, 2'-H) (Found: C, 67.2; H, 5.3; N, 12.7; $C_{18}H_{17}N_3O_3$ requires C, 66.9; H, 5.3; N, 13.0%).

5-Acetamido-1-acetylindol-3-yl Acetate (93)

(a) 1-Acetyl-5-nitroindol-3-yl acetate (200mg) in acetic acid (70ml) and acetic anhydride (10ml) was hydrogenated at room temperature and atmospheric pressure, over Adams catalyst for 2h. Filtration and evaporation gave 1-acetyl-5-acetamidoindoline (94, R=Ac) as a pale yellow solid (81mg, 48.7%), m.p. 213-215° (from ethanol) m/e 218, 176 and 133 (base), ν_{max} 1690 (NAc), 1640(NHAc), 1600, 1537 and 3300cm^{-1} (NH), λ_{max} 275 (ε 25,500), 278(25,300), 297sh(12,300), and 310sh(7840) nm, δ ($CDCl_3$) 2.1 (3H, s, NHAc), 2.2 (3H, s, NAc), 3.1(2H, t, $J_{3,2}$ 8 Hz, 3-H_2), 4.0 (2H, t, $J_{2,3}$ 8 Hz, 2-H_2), 7.0 (1H, d, $J_{5,6}$ 8 Hz, 5-H), 7.4 (1H, s, NH), 7.7 (1H, d, $J_{4,6}$ 2 Hz, 4-H), and 8.1 (1H, 2 x d, $J_{6,5}$ 8, $J_{6,4}$ 2 Hz 6-H) (Found: C, 66.1; H, 6.4; N, 12.8. $C_{12}H_{14}N_2O_2$ requires C, 66.0; H, 6.5; N, 12.8%).

(b) Repetition of experiment (a) but without acetic anhydride gave colourless prisms of 1-acetyl-5-aminoindoline (94, R=H) (35%), m.p. 165-167° (from benzene), m/e 176, 134, and 133 (base), ν_{max} 1625 (CO) and 1590 cm^{-1}, λ_{max} 272 (ε 15,800) and 310sh (3870) nm, δ ($CDCl_3$), 2.1 (3H, s, NAc), 3.05 (2H, t, $J_{3,2}$ 8 Hz, 3-H_2), 3.3br (2H, s, NH_2), 3.95 (2H, t, $J_{2,3}$ 8 Hz, 2-H_2), 6.45 (1H, 2 x d, $J_{6,7}$ 8, $J_{6,4}$ 2 Hz, 6-H), 6.50 (1H, d, $J_{4,6}$ 2 Hz, 4-H), and 8.0 (1H, d, $J_{7,6}$ 8 Hz, 7-H) (Found: C, 68.0; H, 6.9; N, 15.9. $C_{10}H_{12}N_2O$ requires C, 68.2; H, 6.9; N, 15.9%).

(c) Hydrogenation of (69) (130mg) in dimethylformamide (30ml) and acetic anhydride (15ml) over 5% palladium-carbon during 3h gave, after work-up, a dark coloured solid. This was extracted with hot ethanol; the extract was filtered and evaporated to give 5-N-acetoxyacetamido-1-acetylindol-3-yl acetate (95) (40mg, 24.4%), m.p. 141-142° (from ethanol), m/e 332, 290, 274, 232, 230, 190, and 148 (base), ν_{max} 1785 (NOAc), 1760, 1686 (CO), and 1195 cm^{-1} (CO) [PhNAc(OAc) shows ν_{max} 1795, 1684, and 1186cm^{-1}], λ_{max} 246 (ε 25,000), 301 (4330), and 308sh (4220) nm, δ ($CDCl_3$) 2.05 (3H, s, NAc), 2.2 (3H, s, NAc), 2.35 (3H, s, OAc), 2.60 (3H, s, OAc) 7.5 (1H, 2 x d, J 9 and 2 Hz, 6-h) 7.7 (1H, d, J 2 Hz, 4-H), 7.8 (1H, s, 2-H), and 8.55 (1H, d, J 9 Hz, 7-H) (Found: C, 57.8; H, 5.1; N, 8.3. $C_{16}H_{16}N_2O_6$ requires C, 57.8; H, 4.85; N, 8.4%).

(d) Reduction of (69) as in (c) but in dimethylformamide alone and for 12h. gave on work up, a gum. This was treated with acetic anhydride; removal of the reagent left a solid which partly dissolved in hot ethanol. The red residue was characterized as

1,1'-diacetyl-5,5'-azoindole-3,3'-diyl diacetate (97) (100mg, 5.7%), micro-crystals, m.p. 268-270° (from chloroform), m/e 460, 418, 376, 174 and 132 (base), ν_{max} 1760 (OAc), 1700 (NAc), and 1200cm^{-1} (CO), λ_{max} 245sh (ε 22,800), 255 (23,700), 288 (2560), and 298sh (18,700) nm δ (CF_3 -CO_2H) 2.60 (6H, s, 2 x NAc), 2.90 (6H, s, 2 x OAc), 8.20 (2H, s, 2- and 2'-H), 8.4 (2H, 2 x d, J 10 and 2 Hz, 6- and 6'-H), 8.60 (2H, d, J 2 Hz, 4- and 4'-H), and 8.9 (2H, d, J 10 Hz, 7- and 7'-H) (Found: C, 62.5; H, 4.4; N, 12.1. $C_{24}H_{20}N_4O_6$ requires C, 62.6; H, 4.4; N, 12.2%).

On concentration of the ethanolic extract the acetamido-derivative (93) crystallised, giving needles (1.4g. 67%), m.p. 221-223, m/e 274, 232 and 190 (base), ν_{max} 3300 (NH), 1750(OAc), 1710 (NAc), 1660 (NHAc), and 1210 cm^{-1} (CO), λ_{max} 247 (ε 20,900), 300 (4120), and 305 (4000) nm, δ ($CDCl_3$) 2.1 (3H, s, NHAc), 2.35 (3H, s, NAc), 2.55 (3H, s, OAc), 7.4 (1H, 2 x d, J 8 and 2 Hz, 6-H), 7.75 (1H, s, 2-H), 8.1 (1H, d, J 2 Hz 4-H), and 8.45 (1H, d, $J_{7,6}$ 8 Hz, 7-H) (Found: C, 61.3; H, 5.3; N, 10.0. $C_{14}H_{14}N_2O_4$ requires C, 61.3; H, 5.1; N, 10.2%).

Further concentration of the mother liquor from which the amide (93) separated gave 5-acetamido-1-acetylindoxyl (98) (255mg, 14.4%), m.p. 246-247° (from ethanol), ν_{max} 1725(NAc), 1690(CO), and 1645cm^{-1} (NHAc), λ_{max} 249 (ε 27,500), 269sh (17,700), 278(21,500), 286sh (16,800), and 368(3840) nm, δ [$(CD_3)_2SO$] 2.05 (3H, s, NHAc), 2.2 (3H, s, NAc), 2.5 (2H, s, 2-H_2), 7.7 (1H, 2 x d, J 8 and 2 Hz, 6-H), 8.0 (1H, d, J 2 Hz, 4-H), 8.3 (1H, d, $J_{7,6}$ 8 Hz, 7-H), and 10.1br (1H, s, NH) (Found: C, 61.95; H, 5.2; N, 12.1.

$C_{12}H_{12}N_2O_3$ requires C, 62.1; H, 5.2; N, 12.1%).

(E)-5-Acetamido-2-{1-[3-(1-methoxyethyl)-4-pyridyl]ethylidene}indolin-3-one (99).

To a mixture of the indoxyl (93) (2g) and the ketone (59) (1 mol. equiv.) in methanol (60ml) under nitrogen was added potassium hydroxide (15.6g) in water (60ml). The mixture was then sealed up and left for 7 days, and the product was then filtered off under nitrogen; yield 0.7g (27.8%), m.p. 232-234°, m/e 351, 304 (base), 292 and 262, ν_{max} 1673 (CO), 1630 (NAc), and 1605 cm^{-1} (C=C), λ_{max} 269 (ε 22,100), 290sh (14,500), and 488 (3440) nm, δ[$(CD_3)_2SO$](Fig. 19) 1.2 and 1.3 (3H, 2 x d, J 7 Hz, CH.CH_3), 2.1 and 2.15 (3H, 2 x s, C.CH_3), 3.0 and 3.1 (3H, 2 x s, OMe), 4.3 (1H, q, J 7 Hz, CH.CH_3), 7.05 (1H, d, J 8 Hz, 7-H), 7.1 (1H, d, J 5 Hz 5'-H), 7.65 (1H, 2 x d, J 8 and 2 Hz, 6-H), 7.75 (1H, d, J 2 Hz, 4-H), 8.5 (1H, d, J 5 Hz, 6'-H), 8.65 (1H, s, 2'-H), 9.4br (1H, s, NH), and 9.9br (1H, s, NHAc) (Found: C, 68.1; H, 5.95; N, 11.9. $C_{20}H_{21}N_3O_3$ requires C, 68.4; H, 6.0; N, 12.0%). In other experiments an extractive work-up rather than filtration was employed; in such cases a mixture of E- and Z-isomers was isolated which were not separated but used directly.

5-Acetamido-2-{1-[3-(1-methoxyethyl)-4-pyridyl]ethyl}indole (101)

The indolinone mixture (99) and Z-isomer was reduced with sodium borohydride in boiling ethanol and the product dissolved in methanol and treated with hydrogen chloride. Evaporation and crystallization of the residue from acetone and petroleum (b.p. 60-80°)

afforded (101) as prisms (66%), m.p. 222-223°, m/e 337, 308 and 291 (base), ν_{max} 3220 (NH), 1660 (NAc), 1590 and 1545cm^{-1}, λ_{max} 243 (ε 31,200), 301 (9000), and 311 (8300) nm, δ[$(CD_3)_2SO$] (Fig. 27) 1.3 and 1.35 [3H, 2 x d, J 7 Hz, CH(OMe).$\underline{CH_3}$], 1.6 (3H, d, J 7 Hz, CH.$\underline{CH_3}$), 2.0 (3H, s, NHAc), 3.08 and 3.12 (3H, 2 x s, OMe), 4.6 (1H, q, J 7 Hz, $\underline{CH}$.CH_3), 4.8 (1H, q, J 7 Hz, $\underline{CH}$(OMe).CH_3), 6.15 (1H, s, 3-H), ca. 7.2 (3H, m, 6-, 7-, and 5'-H), 7.7 (1H, d, J 2 Hz, 4-H), 8.4 (1H, d, J 5 Hz, 6'-H), 8.5 (1H, s, 2'-H), 9.65 (1H, s, NH), and 10.7 (1H, s, NHAc) (Found: C, 71.0; H, 6.8; N, 12.4. $C_{20}H_{23}N_3O_2$ requires C, 71.2; H, 6.9; N, 12.45%).

9-Aminoellipticine (9-Amino-5,11-dimethylpyrido[4,3-b]-carbazole (92)

The indole (101) (140mg) in aqueous 60% hydrobromic acid (4 ml) was heated under reflux for 18h. The yellow product was then filtered off,* dissolved in water, basified with sodium hydrogencarbonate, and extracted with ethyl acetate. The solvent was removed and the residue was crystallized from benzene to give 9-aminoellipticine benzene solvate (63mg) as yellow prisms, m.p. 255-260° (decomp.). m/e 261 (base), ν_{max} 3125 (NH), 1615, and 1595 cm^{-1} λ_{max} 253 (ε 12,600), 283(36,800), 297.5(42,500), 341(6370), 358sh(4150), and 420(3240)nm, δ($CDCl_3$) (Fig. 28), 2.7 (3H, s, 5-Me), 3.2 (3H, s, 11-Me), 4.8br (2H, s, NH_2), 6.9 (1H, d, J 8 Hz, 8-H), 7.3 (1H, d, J 8 Hz, 7-H), 7.65 (1H, s, 10-H), 7.8 (1H, d, J 6 Hz, 4-H), 8.35 (1H, d, J 6 Hz, 3-H), 9.6 (1H, s, 1-H), and 10.8 (1H, s, NH) [Found (sample dried at 100° for 10h under high vacuum): C, 78.0; H, 5.7; N, 16.1. $C_{17}H_{15}N_3$ requires C, 78.1; H, 5.8; N, 16.1%].

* Basification of the filtrate followed by chloroform extraction furnished ellipticine (21mg) m.p. 309-312° (lit[1] 311-315).

3-Acetylethylene acetal pyridine (106).

3-Acetylpyridine (11g) was heated with a solution of p-toluene sulphonic acid (1.1 molar equivalents) in ethylene glycol (25ml) and dry benzene (400ml) in a Dean Stark apparatus for 8h. The mixture was then cooled, separated and the lower ethylene glycol layer diluted (ice water) basified (Na_2CO_3) and extracted with $CHCl_3$ to give (106). A further quantity was obtained from the benzene layer after washing with sodium carbonate solution drying and evaporating. Distillation at 6.5mm/122° afforded (106). Yield 94%. m/e 165 (M^+).

Attempted Wibaut-Arens reductive acetylation of (106)

The acetal (106) (8.4g) was treated with zinc and acetic anhydride as previously described yielding a viscous orange oil

Heating this material in methanol furnished starting acetal (106) (6g) and a complex orange gum which was unaffected by prolonged thermal degradation.

3-Acetylethylene acetal pyridine-1-oxide (107)

3-Acetylpyridine-1-oxide (4.9g) was reacted with ethylene glycol as previously described for the preparation of (106). The combined yields were distilled, oil bath temperature 200-230°, to give a colourless oil which slowly crystallized as a hygroscopic solid with an indefinite m.p. ν_{max} 2980, 2880, 1030cm^{-1} δ ($CDCl_3$) 3.95 (4H, m, OCH_2CH_2O), 1.65 (3H, s, CCH_3).

The hydrochloride salt of this compound was prepared by treatment with chloroform saturated with hydrogen chloride followed by removal of the solvent.

Reaction of (107) with phosphorus oxychloride.

The hydrochloride salt of 107 (1.66g) was heated with phosphorus oxychloride (6cc) at 120° for 2h. After cooling, the solution was poured onto 2N aqueous potassium carbonate solution and ice; ether extraction then afforded an oil (1.48g) which partially crystallized on standing. A mass spectrum of this product showed a molecular ion cluster m/e 217/219/221 ($C_9H_9NOCl_2$) presumed to be due to compound (110), a group of ions corresponding to $C_9H_{10}NO_2Cl$, probably (108, X = Cl, Y = H), and (108, X = H, Y = Cl) and a further cluster m/e 411/413/415 ($C_{21}H_{12}N_3Cl_3$) due to the tetracycle (109).

The oil was warmed with 6N hydrochloric acid in a water-bath for 10 min. and the solution then extracted with chloroform*. The organic phase was washed with 5% sodium carbonate solution, dried and evaporated to give anoil which crystallized on trituration with ether affording (109) as pale yellow plates (80mg), m.p. 264-6° (acetone), ν_{max}

* The aqueous phase was basified and extracted with chloroform to give an oil (30mg); the H'n.m.r. spectrum of which showed it to be 3-acetyl-2-chloropyridine, ($CDCl_3$)8.50 (1H m, 6-H), 7.92 (1H m, 5-H), 7.35 (1H m, 4-H), 2.70 (3H s $COCH_3$), contaminated with ca 5% of the 6-chloroisomer. Assignment of this latter structure rests upon the fact that only one α-pyridine proton signal is observed (8.95 d, J= 2Hz) the remainder of the spectrum is difficult to assign because of the low intensity of the signals, apart from that due to the acetyl protons which appear as a singlet (δ 2.62).

1600, 1580, 1560, 1205cm^{-1}, λ_{max} (ε) 257(39,640), 278sh (25,280)nm., δ ($CDCl_3$) (Fig. 20), 8.7 (3H d, $\underline{J}$ = 2 Hz, 3 x 6 - $\underline{H}$), 7.95 (3H 2 x d, $\underline{J}$ = 8 Hz, $\underline{J}$ = 2 Hz, 3 x 4 - $\underline{H}$), 7.75 (3H s, benzenoid protons), 7.5 (3H d, $\underline{J}$ = 8 Hz, 3 x 5 - $\underline{H}$) [Found: C, 61.0; H, 3.0; N, 10.1 $C_{21}H_{12}N_3Cl_3$ requires: C, 61.1; N, 2.9; C, 10.2%.]

4-Chloronicotinic acid

4-Chloro-3-picoline (13g), prepared by treating the N-oxide of 3-picoline[97] with phosphorus oxychloride was dissolved in water (500ml) and potassium permanganate (43g) added. After heating under reflux for 2h., the manganese dioxide which had formed was filtered off and the filtrate reduced to 200ml and then acidified with concentrated hydrochloric acid. The crude product was collected and crystallized from water. Yield 48% m.p. 166-7° (lit,[100] 164°).

Action of dimethyl cadmium upon the acid chloride of 4-chloronicotinic acid. 4-Chloronicotinic acid (5.3g) was heated under reflux with thionyl chloride (80cc) for 3h. Excess reagent was then distilled and last traces removed by the addition of benzene and evaporation under reduced pressure. The residue was covered with dry ether and treated with dry triethylamine, after stirring for 10h. the precipitate of triethylamine hydrochloride which formed was removed and the filtrate reacted at room temperature with an excess of dimethyl cadmium in ether. The reaction mixture was then heated under reflux for a further 3h. After cooling, 15% aqueous solution of ammonium chloride was added to decompose the complex and excess dimethyl cadmium, the ether layer

separated and the aqueous phase extracted with ether. The ether layers were then combined, washed with sodium carbonate solution dried and evaporated to give an orange gum. This was dissolved in chloroform* and extracted with 2N. sulphuric acid. The acid extract was then basified to yield 4-chloro-N,N-diethylnicotinamide (144), as an oil (400mg) m/e 212/214 (M^+), ν_{max} 1635cm^{-1} δ ($CDCl_3$) 1.08 (3H, t, J = 7Hz, $CH_2\underline{CH_3}$), 1.26 (3H t, J = 7Hz, $CH_2\underline{CH_3}$), 3.14 (2H q, J = 7 Hz, $\underline{CH_2}CH_3$), 3.60 (2H q, J = 7 Hz, $\underline{CH_2}CH_3$), 7.35 (1H d, J = 5 Hz, 5 - H), 8.45 (1H s, 2 - H), 8.50 (1H d, J = 5 Hz, 6 - H). The oil decomposed when attempts were made to distill it.

* The chloroform layer was dried and evaporated to give a yellow solid (112) which crystallized on trituration with acetone: yellow plates, m.p. 239-41° (acetone) m/e 294/296/298, ν_{max} 3160, 1580, 1105, 1010 cm^{-1} δ (CF_3CO_2H) (Fig. 29) 9.42 (2H d, J = 2 Hz, 2 - H, 2'-H), 9.16 (2H 2 x d, J = 8 Hz, J = 2 Hz, 6 - H, 6' - H), 8.2 (2H d, J = 8 Hz, 5 - H, 5' - H), 7.34 (1H s, =CH-) [Found: C, 53.0; H, 3.0; N, 9.4. $C_{13}H_8N_2O_2Cl_2$ requires C, 52.9; H, 2.7; N, 9.5 %]

5,5-Dimethyl-(pyrido[3,4-c])furan-2-one (122). Cinchomeronic acid (3.8g) was treated with thionyl chloride (20cc) and dimethylformamide (2g.) and the mixture heated under reflux for $2\frac{1}{2}$h. After standing at room temperature overnight, solvent and reagent were removed and the residue treated with a five fold excess of dimethyl cadmium in ether. Benzene was then introduced, the ether distilled off and the reaction mixture held at 36-38° for $3\frac{1}{2}$h. On cooling, a solution of ammonium chloride (20g) in water (100cc) and conc. hydrochloric acid (20cc) were added slowly; the aqueous layer was then separated, basified with sodium carbonate and extracted with chloroform. Removal of the chloroform yielded crude (122) as a red oil which gradually crystallized on trituration with ether. The product (200mg) recrystallized from ether as yellow needles m.p. 152-5° (lit.[106], 160-1°). ν_{max} 1760, 1608, 1300, 1040cm^{-1} / δ ($CDCl_3$) 9.10 (1H s, 2-H), 8.84 (1H d, J = 6 Hz, 6 - H), 7.40 (1H d, J = 6 Hz, 5 - H), 1.65 (6H s, $C(CH_3)_2$) [Found: C, 66.0; H, 5.7; calc. for $C_9H_9NO_2$: C, 66.2; H, 5.6%]

Cinchomeronamide

Dimethylcinchomeronate (6.6g) in methanol (100ml) was cooled in ice and saturated with ammonia gas. After 3 days at 0°, the solvent was partially evaporated causing crystallization of cinchomeronoamide (96% yield) m.p. 150-170° (resolidified and finally remelts at 226-7°). (lit.[113], 175-176° and 226-7°) m/e 165 (M^+) and 148 (base).

3,4-Dicyanopyridine

Preparation

A stirred suspension of cinchomeronamide (3g) in dry dimethylformamide (30ml) was treated with phosphoryl chloride (5ml) and after the initial reaction had subsided the mixture was heated on a steam bath for 3 min. The dark coloured mixture was poured onto ice, basified with ammonium hydroxide (0.880) and extracted with ether. Evaporation of the dried organic phase gave 3,4-dicyanopyridine (yield 98%) m.p. 80-1° (petrol 60-80°) m/e 129 (M^+, Base) and 102, ν_{max} 2220 (C ≡ N)cm^{-1}

Reaction with methyl magnesium bromide.

3,4-Dicyanopyridine (2g) dissolved in dry tetrahydrofuran (THF) (40ml) was treated dropwise with methyl magnesium bromide (5 mole equiv.) also dissolved in THF. The heterogeneous mixture was stirred for 8h at 33° and then evaporated. Hydrochloric acid (50ml of a 5% aqueous solution) was added and the mixture stirred at room temperature for 12h. Basification (Na_2CO_3) and extraction with chloroform furnished an intractible tar.

Reaction of (116, R = Me) with ethyl acetate

Dimethyl cinchomeronate (6g) and ethyl acetate (12g) were added to solvent free sodium methoxide (from 2.2g Na) stirred for 1h. then heated under reflux for 10h. After a further 5h at room temperature, water (75ml) was added and the solution washed with ether. Dissolved ether was removed from the aqueous phase by heating and then the solution was acidified with concentrated hydrochloric acid (50ml).

After heating under reflux for 2½h the black heterogeneous mixture was cooled, basified and extracted with chloroform. Evaporation of the dried organic phase gave a gum which was composed of a number of compounds (TLC).

Reaction of sodium cyanide with 3-acetyl-1-ethoxypyridinium sulphate.

3-Acetylpyridine 1-oxide (1g) was heated with diethyl sulphate (1 mole equivalent) during 2½h. The product in water (3ml) was added dropwise to potassium cyanide (1.5 molar equivalents) in water (5ml) at 45° in 15min. and the reaction mixture stirred for another ¾h. then extracted with water and chloroform. Evaporation of the dried organic phase furnished a multi-component red-brown gum.

3-Acetyl-4-cyano-1,4-dihydro-1-benzyl pyridine (127) [109(a)]

3-Acetyl-1-benzyl pyridinium bromide (3g) in water (80cc) was added dropwise in 30 min. to a vigorously stirred solution of potassium cyanide (5.8g) in water (20cc). After a further 30min, the yellow solid which had formed was collected by filtration, and crystallized from ethanol. Yield 79.5% m.p. 111-12° (lit.[109(a)], 114-15°).

Attempted aromatization of (127).

(a) Catalytic dehydrogenation

A solution of (127) (200mg) in benzene was heated to reflux in the presence of 5% palladium on carbon for 12h. Extraction with dilute hydrochloric acid, basification and re-extraction with chloroform gave a gum (4.3mg). The organic phase was dried and evaporated to give crude starting material. Repetition of the above procedure using either m-xylene or nitrobenzene instead of benzene gave similar results.

with thionyl chloride

Thionyl chloride (30mol. equiv.) was added dropwise over 30 min. to a stirred solution of (127) (140mg) in benzene maintained at 35-40°. A gum collected on the sides of the flask from which the solvent was decanted, TLC analysis of this product suggest it to be a complex mixture.

with iodine

Iodine (615mg) dissolved in ethanol (15ml) was added dropwise to a hot (70°) ethanolic solution of (127) containing sodium acetate (123mg). The solvent was decanted from the gum which formed and the latter was washed with ethanol (5ml), but again the product was complex.

with dichloro-dicyano-p-benzoquinone (DDQ).

Solutions of (127) (160mg) and DDQ (1 mole equiv.) in benzene were mixed and stirred 20 min at RT. A green solid was filtered off and dissolved in the minimum of acetone and treated with aqueous KI solution. This caused the precipitation of a yellow-brown solid (270mg). Attempts to crystallize this material failed, but infra-red analysis showed it to be devoid of cyano groups.

3-Acetyl-4-cyano-1,4-dihydro-1-(4-nitrobenzyl)pyridine (129).

This was prepared by the application of the conditions used in the synthesis of (127). The product was an unstable yellow solid. m.p. 110-30° (dec.) ν_{max} 2230, 1680, 1640, 1580, 1520, 1340 cm^{-1}. δ (d_6-DMSO) 8.65 (2H d, J = 9 Hz, 3'-H, 5'-H), 8.24 (1H s, 2-H), 7.96 (2H d, J = 9 Hz, 2'-H, 6'-H), 6.70 (1H d, J = 8 Hz, 6 - H), 5.25 (1H 2 x d, J = 8 Hz, J = 5 Hz 5 - H), 5.02 (2H s, CH_2Ar), 4.72 (1H d, J = 5 Hz, 4 - H), 2.32 (3H s, $COCH_3$).

Reaction of (129) with hydrochloric acid. The dihydropyridine (114mg) in chloroform (7cc) was treated with 2N aqueous hydrochloric acid (10cc) and the reaction mixture heated at 55-60° for 1h. The aqueous phase was separated neutralized with sodium carbonate and extracted with chloroform to yield a gum which crystallized from ethanol to give (130) as pale yellow prisms, m.p. 179-80° (90.6mg), m/e 319, 275(B), ν_{max} 3300, 1660, 1620, 1570, 1510, 1345 and 1060cm^{-1}. δ (d_6 - DMSO) (Fig. 21) 8.22 (2H d, J = 8 Hz, 3'-H, 5'-H), 7.85 (1H s, 2-H), 7.58 (2H d, J = 8 Hz, 2'-H, 6'-H), 7.5 (1H bs, OH), 7.36 (2H bd, J = 14 Hz, $CONH_2$), 4.76 (2H s, CH_2Ar), 4.52 (1H d, J = 10Hz, CHOH), 3.65 (1H 2 x d, J = 6 Hz, J = 2 Hz, 4 - H), 2.20 and 1.82 (2H m, 5-H_2), 2.12 (3H s, $COCH_3$). [Found: C, 56.3; H, 5.4; N, 13.0 $C_{15}H_{17}N_3O_5$ requires: C, 56.4; H, 5.4; N, 13.2%.]

Reaction between 3-acetylpyridine and hydroxylamine-O-sulphonic acid

3-Acetylpyridine (1.94g) in water (4cc) was added to a solution of hydroxylamine-O-sulphonic acid (3.62g) and potassium hydroxide (1.79g) in water (6.4cc) maintained below 0°. After the addition the temperature was raised to 70° for 4h, the solution then cooled and basified with sodium carbonate (2.2g) in water (3.3cc). After ½h. the reaction mixture was cooled in ice , acidified with conc. hydrochloric acid, filtered and the filtrate evaporated. The residue was treated with methanol and filtered again. On concentration, the methanol filtrate yielded a crystalline solid. Without purification, this was reacted with acetic anhydride (16cc) during 5h., excess reagent was then removed under reduced pressure to afford a brown oil which was chromatographed on neutral alumina

(100g) using 2% methanol in dichloromethane. Fifty 30cc fractions were collected: TLC indicated that fractions 3-34 were identical and on combination and evaporation these yielded a solid which crystallized from di-isopropyl ether/60-80° petrol as pale yellow prisms (0.99g) m.p. 116-7°. This material proved to be identical with the oxime of 3-acetylpyridine (lit.,[114] m.p. 113°).

The remaining fractions were combined and evaporated to yield (136) as a colourless solid (30mg) m.p. 188-90° (acetone) m/e 193 ν_{max} 3400-2500, 1560, 1030cm^{-1}., δ(d_6-DMSO), 8.90 (1H d, J = 2 Hz, 2 - H), 8.70 (1H m, 6-H), 8.38 (1H m, 4-H), 7.9 (1H m, 5-H), 2.20 (3H s, CH_3CO), 1.85 (3H s, CH_3C). (Found: C, 56.0; H, 6.1; N, 22.05. $C_9H_{11}N_3O_2$ requires C, 55.95; H, 5.7; N, 21.75%).

3-Acetylethyleneacetal-1-aminopyridinium mesitylenesulphonate.

3-Acetylethylene acetal pyridine (15.6g) in dichloromethane (42 cc) was cooled to 0° and a solution of O-mesitylene sulphonyl hydroxylamine (20.4g) in dichloromethane (40cc) added. After stirring at room temperature for 30 min., the solution was diluted with 800cc of diethyl ether and again cooled to 0°. After a few minutes, the crystalline product was collected (33.8g 94.0%); m.p. 118-9°, ν_{max} 3210, 3130, 1190cm.$^{-1}$ δ(d_6-DMSO) 9.15 (1H s, 2-H), 9.10 (1H d, J = 8 Hz, 6-H), 8.95 (2H bs, NH_2), 8.50 (1H d, J = 9 Hz, 4 - H), 8.35 (1H q, J = 8 Hz, J = 9 Hz, 5 - H), 7.03 (2H s, benzenoid protons) 4.3-3.8 (4H m, OCH_2CH_2O), 2.50 (6H s, 2 x CH_3) 2.49 (3H s, CH_3), 1.70 (3H s, CH_3).

3-Acetylethylene acetal-1-(N-acetyl)pyridine. The product (33g) from the previous reaction was dissolved in water (100cc) and treated with acetic anhydride (200cc), previously cooled to 5°, and then drop-wise with 30% aqueous sodium hydroxide solution (150cc).

The mixture was then poured into a solution of potassium carbonate (100g) in water (900cc) and stirred with chloroform (100cc). A colourless precipitate which formed was removed and the aqueous phase separated and extracted with chloroform (100cc). The chloroform layers were combined, dried and evaporated to give (136, X = OCH_2CH_2O) as an amber coloured oil which slowly crystallized as a hydroscopic solid (19.3g 98%). m/e 222, 207 (B), ν_{max} 1570cm^{-1} $\delta(CDCl_3)$ 9.0 (2H m, 2-H, 6-H), 8.35 (1H d, J = 8 Hz, 4-H), 7.95 (1H q, J = 8 Hz, J = 7Hz, 5 - H), 4.40-3.90 (4H m, OCH_2CH_2O), 2.13 (3H s, $COCH_3$), 1.72 (3H s, CH_3).

3-Acetylethyleneacetyl-1-(N-methyl acetamido)pyridinium iodide (138)

The pyridine (136, X = OCH_2CH_2O) (15.1g) was reacted with methyl iodide (150cc) at reflux during 45min. Removal of excess reagent afforded a yellow solid (98% Yield) m.p. 176-7° (EtOH).

3-Acetylethyleneacetal-4-cyanopyridine (140). The salt (138) (24g) in water (56ml) was warmed to 20-22° and treated with ammonium chloride (7.0g) and potassium cyanide (5.6g) in water (10ml). After 1h., the mixture was extracted with chloroform to yield an oil which when chromatographed upon basic alumina (400g) eluting with ether gave (140) as a colourless solid which recrystallized from ethyl acetate in the form of needles. Yield 10.2g (81.5%) m.p. 68-9° m/e 190, 175 (B), ν_{max} 2220 (C ≡ N) and 1035 (C - O)cm^{-1}. δ (d_6 - DMSO)

1.70 (3H s, $\underline{CH_3}C$), 3.9 (4H m, $\underline{CH_2}\underline{CH_2}$), 7.8 (1H d, $\underline{J}$ = 5 Hz, 5 - $\underline{H}$), 8.7 (1H d, $\underline{J}$ = 5 Hz, 6 - $\underline{H}$), 8.8 (1H s, 2-$\underline{H}$), (Found: C, 63.2; H, 5.3; N, 14.7; $C_{10}H_{10}N_2O_2$ requires: C, 63.15; H, 5.3; N, 14.7%).

4-Acetyl-3-acetylethylene acetal pyridine (105).

The nitrile (140) (1.0g) in ether (30cc) was added slowly to a solution of methyl lithium (1.2 mol. eq.) in ether at -10-15° under an atmosphere of nitrogen. Stirring was maintained for a further period of 30min. and then ice-water (40cc) was introduced followed by ammonium chloride (1.0g) in water (10cc).

The ether layer was removed, dried and evaporated to give a small amount of starting material. Extraction of the aqueous phase with chloroform gave an oil which slowly crystallised to colourless prisms of the imine (141); m.p. 85-6° (ether), 0.97g (89.5%). ν_{max} 1640, 1590 cm^{-1}, $\delta(CDCl_3)$ 1.8 (3H s, $\underline{CH_3}C$), 2.40 (3H s, $\underline{CH_3}$ C=N), 3.7-4.1 (4H m, $O\underline{CH_2}\underline{CH_2}O$), 7.01 (1H d, $\underline{J}$ = 5.5 Hz, 5 - $\underline{H}$), 8.55 (1H, d, $\underline{J}$ = 5.5 Hz, 6 - $\underline{H}$), 8.82 (1H s, 2-$\underline{H}$), (Found: C, 64.0; H, 6.7; N, 13.3. $C_{11}H_{14}N_2O_2$ requires C, 64.1; H, 6.8; N, 13.6%).

Treatment of the imine (5.7g) with 20% aqueous acetic acid (160cc) on the steam-bath for 30min. followed by basification (K_2CO_3) and extraction with chloroform afforded the 4-acetyl derivative (105) as colourless prisms 5.3g (94.5%), m.p. 49-50° (from 60-80° b.p. petrol). $\underline{m/e}$ 207 (v.weak), 206 and 191 (Base), ν_{max} 2930, 2890, 1705, 1030 cm^{-1}; λ_{max} 263(2,490) nm. $\delta(CDCl_3)$ (Fig. 30) 1.8 (3H s, $\underline{CH_3}C$), 2.5 (3H s, $\underline{CH_3}CO$) 3.8 (4H m, $O\underline{CH_2}\underline{CH_2}O$), 7.0 (1H d, $\underline{J}$ = 5 Hz 5-$\underline{H}$), 8.55 (1H d, $\underline{J}$ = 5 Hz, 6-$\underline{H}$), 8.75 (1H s, 2-$\underline{H}$), (Found: C, 63.7; H, 6.4; N, 6.7; $C_{11}H_{13}NO_3$ requires: C, 63.75; H, 6.3; N, 6.8%).

3,4-Diacetylpyridine (142)

Hydrolysis of the acetal (105) with 20% aqueous hydrochloric acid at 100° for 30 min, gave 3,4-diacetyl pyridine as a colourless solid, m.p. 42-4° (40-60° petrol) (Yield 96%) λ_{max} (ε) 227(5,740), 275(2,420)nm ν_{max} 1710 (broad), 1590cm^{-1}. δ($CDCl_3$) 2.50 (3H s, $\underline{CH_3}CO$),2.64 (3H s, $\underline{CH_3}CO$), 7.32 (1H d, J=6 Hz, 5-H), 8.85 (1H d, J=6 Hz, 6-H), 9.05 (1H s 2-H). (Found: C, 66.0; H, 5.7; N, 8.9. C_9H_9NO requires: C, 66.2; H, 5.6; N, 8.6%.)

Reaction between 3,4-Diacetylpyridine and ethan-1,2-diol. 3,4-Diacetylpyridine (1.6g) in dry benzene (50cc) containing p-toluene sulphonic acid (1.15 molar excess) and ethan-1,2-diol (5cc) was heated in a Dean-Stark apparatus for 12h. Removal of the solvent gave a red oil which was chromatographed upon basic alumina (50g), eluting with ether. Thirty 50cc fractions were collected. Fractions 1-7 yielded a white crystalline solid (64.mg) m.p. 141-2 (60-80° petrol) to which structure (145) is allocated. m/e 207, 147 (B) λ_{max} (ε) 260 (1,750), 265sh(1,520)nm. δ($CDCl_3$) 1.80 (3H s, $\underline{CH_3}C$), 1.83 (3H s $\underline{CH_3}C$), 3.3 and 3.85 (2 x 2 H m, $O\underline{CH_2}\underline{CH_2}O$), 7.25 (1 H m, 5-H), 8.62 (2H m 2-H, 6-H), (Found: C, 63.8; H, 6.8; N, 7.2. $C_{11}H_{13}NO_3$ requires: C, 63.75; H, 6.3; N, 6.8%).
Fractions 23-30 gave an oil (70mg) the H' nmr of which indicates a mixture of the acetals (143) and (105) in the proportions of about 3:1 to be present.

Reaction between 1,3-diacetylindoxyl and 3,4-diacetylpyridine

1,3-Diacetylindoxyl (1.2g) and 3,4-diacetylpyridine (0.89g) in deoxygenated methanol (10cc) were treated with a solution of potassium hydroxide (3.5g) in deoxygenated water (10cc) and the reaction mixture protected by an atmosphere of nitrogen. After 3 days at room temperature the solution was poured onto ice and 10% aqueous acetic acid; it was then basified with sodium carbonate, extracted with chloroform and the extracts evaporated to yield a deep red coloured oil. This was

chromatographed on alumina eluting with benzene-chloroform mixtures and the major fraction (650mg) was evaporated and the residue triturated with ethanol to give (146) as red prisms. This product does not have a definite m.p., but begins to darken at 290°. m/e 260 (B), 245, λ_{max} (ε) 229 (8,230), 240(8,110), 290(8,790), 336(6,760), 356sh (4,510), 542(6,310), 612(3,610)nm. ν_{max} 1690, 1630, 1610, 1600cm^{-1}, $\delta(CF_3CO_2H)$ (Fig. 22), 9.2 (1H d, J=7 Hz) 8.6–8.45 (m 2H), 8.0–7.6 (m 4H), 7.4–7.1 (m 3H), 246 (s 3H), (Found: C, 78.4; H, 4.6; N, 10.8; $C_{17}H_{12}N_2O$ requires: C, 78.4; H, 4.65; N, 10.8%.)

3-[1-(2-Tetrahydropyranyloxy)ethyl]pyridine (151). The hydrochloride salt of 3-(1-hydroxy)ethyl pyridine (32.4g) was reacted with 2,3-dihydropyran (3 mol.eq.) in dimethylformamide (200cc) and dry hydrogen chloride was then bubbled through the solution for 10min. The reaction mixture then was set aside for 5 days. After removal of the solvent under reduced pressure the residue was treated with a mixture of ether and 10% aqueous sodium carbonate solution. The organic layer was then separated, washed with water, dried and evaporated to give the required ether as a colourless oil, 37.8g (90.0%), b.p. 100–2°/0.65mm.

This product was converted, by a similar series of steps to those described previously for (105), into 4-acetyl-3[1-(2-tetrahydropyranyloxy) ethyl]pyridine (149) which was obtained as a colourless oil, after chromatography upon alumina and elution with ether. Yield from 3-(1-hydroxy)ethylpyridine (65%). m/e 249, ν_{max} 1700, 1580, 1255cm^{-1}. $\delta(CDCl_3)$ (Fig. 31) 9.04 (1H 2 x s, 2-H), 8.75 (1H m, 6-H), 7.4 (1H m, 5-H), 5.3 (1H m, $\underline{CH}CH_3$), 3.96–3.31 (3H m, -CH-O, $-\underline{CH_2}O$), 2.63 (3H s, $\underline{CH_3}CO$), 1.8–1.3 (9H m $\underline{CH_2}\underline{CH_2}\underline{CH_2}$, $CH\underline{CH_3}$) (Found: C, 67.3; H, 7.5; N, 5.8 $C_{14}H_{19}NO_3$ requires C, 67.4; H, 7.7; N, 5.6%.)

Pyrido[3,4c]-2H,5H-2-hydroxy-2,5-dimethyl furan (152).

The 4-acetylpyridine (149) (1.4g), in dilute hydrochloric acid (20cc) was warmed on the steam bath for 10min. The solution was then basified

with sodium carbonate and extracted with chloroform; removal of the solvent gave (152) as an almost colourless oil. (1.0g). This was purified by repeated chromatography upon alumina, eluting with ether. m/e 165 150 (B), ν_{max} (liquid film) 3300, 1605cm^{-1}. $\delta(CDCl_3)$ 8.44 (1H two overlapping doublets, 2-H), 8.34 (1H m, 6-H), 7.3 (1H m, 5-H), 5.36 (1H two overlapping quartets $\underline{CH}CH_3$) 1.78, 1.78 (3H two singlets, $\underline{CH_3}C$), 1.50 (3H two overlapping d, $\underline{CH_3}CH$), 4.92 (1H bs OH), λ_{max} 255sh (1, 130), 250 (1,320), 266sh(1,130)nm. (Found: C, 65.0; H, 6.3; N, 8.2. $C_9H_{11}NO_2$ requires C, 65.4; H, 6.7; N, 8.5%)

(E)- and (Z)-2-{{1-{3-[1-(2-Tetrahydropyranyloxy)ethyl]-4-pyridyl}ethylidene}indolin-3-one (153 and 154, R=H) 1,3-Diacetylindoxyl (750mg) and (149) (860mg) in deoxygenated methanol (6.5cc) were treated with potassium hydroxide (2.5g) in deoxygenated water (6.5cc) and the reaction mixture stored for four days under a protective atmosphere of nitrogen. The solid product was collected by filtration under nitrogen to yield the mixed isomers (0.93g, 74.5%). m/e 364, 280, 247 (B), λ_{max} (ε) 239(21,210), 264(32,270), 296sh(14,440), 463(7,447)nm. This material was not purified further.

2-(pyridyl[3,4b]-3H, 5H-2,5-dimethylfuran-2-yl)indole(156)

The mixed indolinones (153 R=H) and its (Z)-isomer (300mg) in 20% aqueous ethanol (20cc) were treated with sodium borohydride (300ml) and, after the addition, the orange coloured reaction mixture was warmed on the water bath for 30min. The solvent was then removed to afford a gum which was dissolved in methanol and saturated with hydrogen chloride. Removal of the solvent, basification of the residue with aqueous sodium carbonate and chloroform extraction gave a sticky solid; this was chromatographed on alumina, eluting with 1:1 60-80° petrol:ether to yield (156) as a cream colcured solid, m.p. 178°(benzene). m/e 264, 249(B), λ_{max} (ε) 220(21,600), 268(5,800), 284(4,970), 293(4,200)nm. ν_{max} 1595cm^{-1}. $\delta(CDCl_3)$ (Fig 32) 1.62(3H d, J=6 Hz, $\underline{CH_3}CH$), 1.95(3H s, $\underline{CH_3}C$), 5.4(1H q, J= 6 Hz, $CH_3\underline{CH}$), 6.20(1H d, J=2Hz, 3-H), 6.95-7.60(5H m, 4-H,5-H,6-H,7-H,

5'-H), 8.35 (1H s, 2' - H), 8.45 (1H d, J = 6 Hz, 6' - H), [Found: C, 77.4; H, 5.9; N, 10.3 $C_{17}H_{16}N_2O$ requires: C, 77.25; H, 6.1; N, 10.6%].

2-{1-[3-(1-Hydroxyethyl)-4-pyridyl]ethyl}indole (155). The mixture of indolinones (153, R = H) and its (Z)-isomer (300mg) in 20% aqueous ethanol (20cc) was heated at reflux under a nitrogen atmosphere and treated with sodium borohydride (500mg) in small portions. After 1h., the solvent was removed and the residue partitioned between chloroform and water.

The organic phase was dried, charcoaled and evaporated yielding a green gum. This was dissolved in methanol and saturated with hydrogen chloride for 13 min. Removal of the solvent and partitioning the residue between sodium carbonate solution and chloroform furnished (155) as an almost colourless solid, 217 mg (72.2%), m.p. 208-10° (ethanol) m/e 266, 233(B), λ_{max} (ε) 221 (50,210), 265sh (12,290), 270 (12,640), 284 (11,410), 293 (9,830) nm. ν_{max} 3330, 3100, 1600 cm^{-1}. δ(d_6 - DMSO) 8.52 (1H s, 2'-H), 8.24 (1H d, J = 5Hz, 6'-H), 7.4 - 6.8 (5H m, 4 - H, 5 - H, 6 - H, 7 - H, 5' - H), 6.12 (1H bs, 3 - H), 5.3 (1H bs, OH), 5.15 (1H m, CH_3CHO-), 4.62 (1H q, J = 6 Hz, CH_3CH) 1.62 (3H d, J = 8 Hz, CH_3CHO-), 1.38 (3H d, J = 6 Hz, CH_3CH) [Found: C, 76.6; H, 6.8; N, 10.3. $C_{17}H_{18}N_2O$ requires: C, 76.7; H, 6.8; N, 10.5%].

Ellipticine

Treatment of the indole (155) with boron trifluoride in ether at reflux in the course of 1h gave a yellow gum which contained traces of ellipticine. Repetition of this reaction, but for longer periods, gave complex mixtures; whereas at room temperature only starting material was obtained.

With phosphorus oxychloride in pyridine at room temperature (155) gave 5,11-dihydroellipticine which on standing, particularly in the presence of aqueous acid or during chromatography on silica, gradually oxidized to ellipticine. The total yield of the latter product, however, was poor, 15 - 20%, and we were unable to obtain satisfactory analytical data for the dihydrocompound.

When the indole (155) (30mg) in dichloromethane (2cc) was stirred with active manganese dioxide (300mg) for 12h a yellow solution was obtained. Filtration and evaporation of the solvent gave mainly starting material but extraction of the manganese dioxide with hot ethanol afforded a small quantity (3mg) of ellipticine.

The indole (155) (207mg) in dry dimethyl sulphoxide (1.75cc) was treated with acetic anhydride (1.1cc) and the solution stirred at room temperature for 2 days. After this time the reaction mixture was poured into water (50cc) and treated with excess potassium carbonate. A yellow solid gradually formed and this was collected, dried and washed with hot benzene* to yield ellipticine (130mg).

* Evaporation of the washings gave a small quantity of a brown gum which from mass spectral analysis (m/e 308) probably contained the O-acetate derivative (158).

m.p. and mixed m.p. 309-312° (lit.,[1] 309-313) [Found: C, 83.0; H, 5.6; N, 11.2. Calc. for $C_{17}H_{14}N_2$: C, 82.9; H, 5.7; N, 11.4%].

Spiro-2-(3,6-Dimethyl-3H,6H-pyrido[4,3c]dihydropyran)-2-indolin-3-one (157)

The indole (155, R = NHAc) (50mg) in glacial acetic acid (1cc) containing potassium dichromate (40mg) and water (0.5cc) was heated at 100° for 1h. and then poured onto 2N sodium carbonate solution to yield a gum. This was extracted into chloroform washed with water, dried and the solvent removed to yield a product which crystallized on trituration with acetone as pale yellow needles. Yield 5mg m.p. 244-6 (acetone), m/e 280, 252(B), ν_{max} 1710cm^{-1} λ_{max} (ε) 236 (18,500), 262 (5,650), 410 (2,330)nm. δ(d_6-DMSO) (Fig. 23) 0.98 (3H d, J = 3.5 Hz CH_3CH-), 1.55 (3H d, J = 3.7 Hz, CH_3CHO-) 3.4 (1H q, J = 3.5Hz, CH_3CH-), 5.1 (1H q, J = 3.7 Hz, CH_3CHO-), 6.8 - 7.2 (5H m, C_6'-H and benzenoid protons,) 7.8 (1H s NH), 8.5 (2H bs, C_3'-H, C_5'-H) [Found: C, 72.6; H, 5.9; N, 9.8 $C_{17}H_{16}N_2O_2$ requires: C, 72.8; H, 5.75; N, 10.0%].

(E)- and (Z)-5-Acetamido-2-{{1-{3-[1-(2-tetrahydropyranyloxy) ethyl]-4-pyridyl}ethylidene}}indolin-3-one (153, R = NHAc) and isomer.

5-Acetamido- 1,3-diacetylindoxyl was reacted with the acetyl pyridine (149) in the same manner as previously described, however, in this case the reaction mixture was stored at 15-16° for seven days prior to work up. The product was shown to be a mixture of the required isomers, plus some unacetylated materials. Consequently it was dissolved in ethanol and treated with excess acetic anhydride. After shaking at room temperature for 10 min., the excess reagent

was decomposed with ice-water and the reaction mixture basified with potassium carbonate. Chloroform extraction gave (153, R = NHAc) and its isomer as a red solid (80.0%) M^+ 421. λ_{max} (ε) 266 (14,000), 486 (4,600) nm.

5-Acetamido-2-{1-[3-(1-hydroxyethyl)-4-pyridyl]ethyl}indole (155, R = NHAc) This product, small colourless prisms from aq. ethanol, m.p. 244-5°, was obtained from (153, R = NHAc) and its (Z)-isomer in the same way as described for (155, R = H). Yield 75%. m/e 323, 290 (B), λ_{max} (ε) 242 (22,200), 300 (4,00), 311sh (2,600) nm. ν_{max} 3380 (broad), 1660 (broad), 1600 cm^{-1}. δ(d_6-DMSO (Fig. 33) 8.64 (1H bs, 2'-H), 8.35 (1H m, 6'-H), 7.75 (1H s, 4-H), 7.2 (3H m, 6-H, 7-H, 5'-H), 6.1 (1H bs, 3-H), 5.33 (1H m, OH), 5.2 (1H m, CH_3CHO), 4.6 (1H 2 x q, CH_3CH), 1.6 (3H d, J = 6 Hz CH_3CHO), 1.4 (3H 2 x d, CH_3CH) [Found: C, 70.8; H, 6.5; N, 12.6; $C_{19}H_{21}N_3O_2$ requires: C, 70.6; H, 6.6; N, 13.0%].

9-Acetamidoellipticine (159) When the indole (155, R = NHAc) was treated with dimethyl sulphoxide and acetic anhydride as previously described 9-acetamidoellipticine, m.p. 245-50° (dec) (yellow needles from ethanol) was obtained in 65.2% yield. m/e 303(B), 261, λ_{max} (ε) 225 (6,4600), 257 (10,200), 268sh (10,920), 277 (13,040), 298 (18,620), 308 sh (12,040), 339 (2,480), 354 (1,990) 412 (1,650) nm. ν_{max} 1660, 1600 cm^{-1}. δ(CF_3CO_2H) 9.75 (bs 2H, 1-H, 3-H), 8.65-8.2 (m 3H, 7-H, 8-H, 10-H), 7.75 (s 1H, 4-H), 3.40 (s 3H, 11-CH_3), 2.95 (s 3H, 5-CH_3), 2.68 (s 3H, $NHCOCH_3$) [Found: C, 75.0; H, 5.6; N, 13.9 $C_{19}H_{17}N_3O$ requires: C, 75.2; H, 5.65; N, 13.85%].

PART III

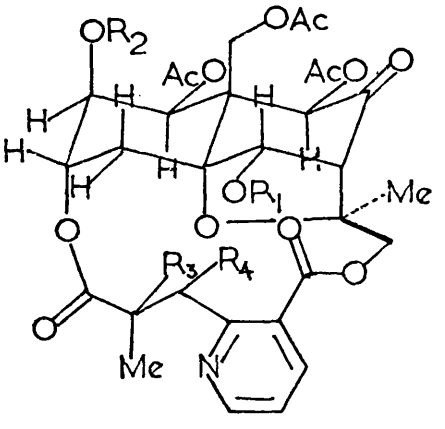
PART III

Introduction

The Celastraceae consists of approximately 60 genera comprising 850 species which occur widespread both as wild and cultivated shrubs and trees. Unlike the Apocynaceae few of the species are alkaloid producers but, from the few genera which have been studied they are generally abundant in terpenoids, flavanoids and related compounds.

However, from the genera: Euonymus[115], Triptergium[116] and Maytenus[16] some novel macrolide compounds (see fig. 34) have been isolated and interestingly the recently isolated alkaloid maytansine (5) from Maytenus species, has been shown to be the most active anti cancer agent known to date[15].

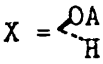
Fig. 34



Evonine R_1 = Ac, X = O, T_2 = Ac, R_3 = H, R_4 = Me

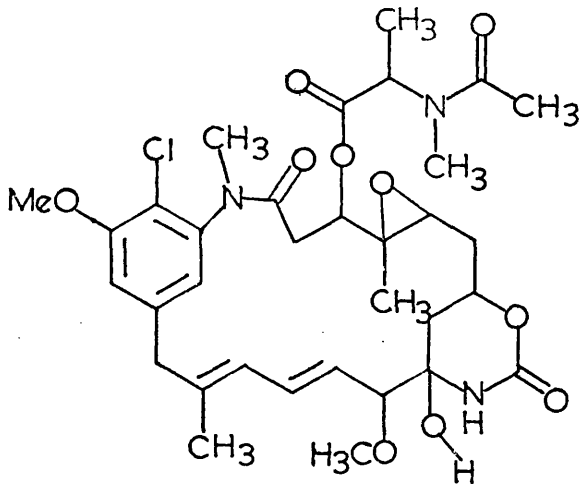
neo-Evonine R_1 = H, X = O, R_2 = Ac, R_3 = H, R_4 = Me

Evonymine R_1 = Ac, X =  , R_2 = Ac, R_3 = H, R_4 = Me

neo-Evonymine R_1 = H, X =  , R_2 = Ac, R_3 = H, R_4 = Me

Wilfordine R_1 = Ac, X =  , R_2 = COPh, R_3 = OH, R_4 = Me

Alatamine R_1 = Ac, X = O, R_2 = COPh, R_3 = OH, R_4 = Me



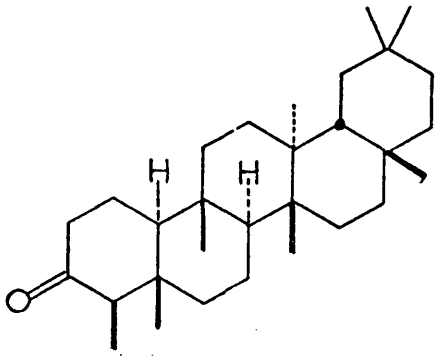
(5)

Maytansine (5) occurs in minute concentrations (approximately 0.2 p.p.m.) in the plant, but the other components (see fig. 34) are much more abundant. Thus attempting to establish a better source of maytansine or a similarly active natural product we undertook a study of a related species: Lophopetalum rigidum Ridley.

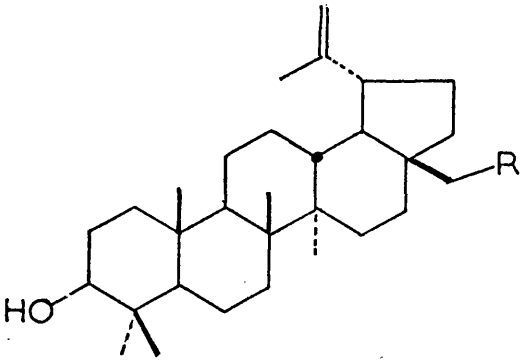
Discussion of Results

The finely divided bark of Lophopetalum rigidum was extracted in succession with petroleum ether (60-80°) and methanol. Contained in the former extract were several known triterpenoids which were separated by chromatography over silica; these are listed in figure 35 . In addition to these common natural compounds we also isolated an aliphatic ester of high molecular weight (approx. 920) and a mixture of n-alkanes (C_{14}-C_{20}).

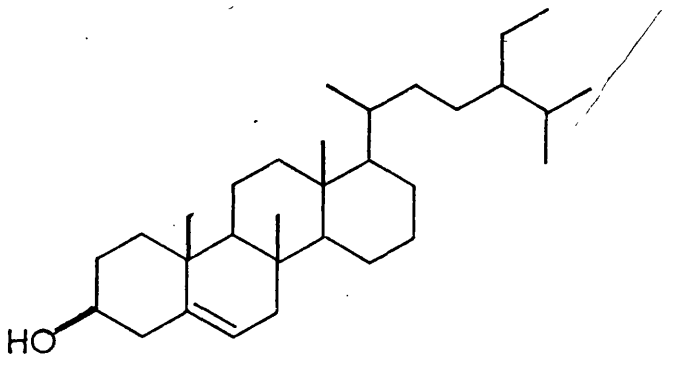
Fig.35



Friedelin



Lupeol R=H
Betulin R=OH



β-Sitosterol

The methanolic extract was condensed in-vacuo to a deep red, sweet smelling gum which dissolved readily in water, alcohol and acetone. Paper chromatography of the crude material was disappointing and of the several solvent systems which we employed only the Partridge system (organic phase of a mixture of n-butanol-acetic acid-water, 4:1:5) showed any separation of definite components, the others merely giving rise to a continuous streak from base line to solvent front on the chromatograms.

Thus we were not surprised to find that column chromatography over cellulose was able to effect only very superficial separation of the components. Furthermore an endeavour to purify the crude fractions by means of preparative paper chromatography was unsuccessful and finally we decided to abandon our work on this extract.

Experimental to Part III

A sample of the bark was transformed into a coarse powder (950g) using a hammer mill and extracted by the Soxhlet technique first with petrol (60-80°) (10l) -A and then with methanol (10l) -B.

Extract A

Evaporation of the solvent furnished a yellow-green gum (16.5g). By chromatographing this material over silica (500g), eluting with petrol (60-80) - chloroform and chloroform-methanol mixtures, five fractions were obtained:

I (400ml of 10% $CHCl_3$-petrol (60-80°))

A pale yellow solid was obtained on removal of the solvent which crystallized from ethyl acetate as a colourless microcrystalline solid (4.3mg) m.p. 82-3° (EtAc), R_f (SiO_2 - 5% $CHCl_3$-C_6H_6) 0.79 (pink colouration by spraying with H_2SO_4 and baking) m/e 920 (M^+), ν_{max} 1730cm^{-1} (ester). Mother liquor on evaporation afforded a brown sweet smelling oil, G.L.C. - MS. analysis of which shows the presence of the n-alkanes C_{14}-C_{20} (confirmed by direct comparison with a synthetic mixture).

II (1900ml of 30% $CHCl_3$-petrol (60-80°))

The solvent free fraction was an off-white solid which when crystallized from ethyl acetate afforded friedelin (1.3g) as colourless needles, m.p., m.mp. 256-7° (lit[117] 261-64°) $[\alpha]_D^{20}$ - 20.6 ($CHCl_3$), R_f(SiO_2 5% $CHCl_3$ - C_6H_6) 0.21 (visuallized by baking with H_2SO_4) m/e 426 (M^+) (Found: C 84.3; H, 11.7. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%).

III (2400 ml 80% $CHCl_3$-petrol (60-80°))

Trituration of the solvent-free residue with ether afforded a further quantity of friedelin (310mg) and from the mother-liquor lupeol (4.68g) was obtained, m.p. 206-8° (MeOH) (lit.[118] 213°), $[\alpha]_D^{20}$ + 25.2 ($CHCl_3$), R_f(SiO_2, 7:3 benzene-ethyl acetate) 0.75 (purple colouration on treatment with hot H_2SO_4), m/e 426 (M^+). (Found: C, 84.3; H, 11.7; Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%). Acetate m.p. 214° from HOAc - Ac_2O (lit[118] 211-212) (Found: C, 82.0; H, 11.2. Calc. for $C_{32}H_{52}O_2$: C, 82.0; H, 11.2%).

IV (1200ml, $CHCl_3$)

The solvent-free residue was washed with methanol and filtered.* Further separation of the solid material was achieved by washing with hot methanol giving friedelin (95mg, total yield 0.18%) and lupeol (315mg).

*Partial evaporation of the solvent caused crystallization giving a further quantity of lupeol (130mg, total yield 0.54%). Total evaporation of the mother-liquor followed by crystallization from aqueous methanol gave β-sitosterol (0.003%) m.p., m.m.p. 135-7° (lit.[119] 137-8°), $[\alpha]_D^{20}$ - 38.3° ($CHCl_3$). (Found: C, 84.0; H, 12.3. Calc. for $C_{29}H_{50}O$: C, 84.0; H, 12.15%).

V (1600ml 5 - 60% MeOH - $CHCl_3$).

Removal of the solvent and crystallization of the residue from aqueous methanol gave betulin (0.013%) m.p., m.m.p. 250-3° (lit.[118] 252°) $[\alpha]_D^{20}$ + 20.3 ($CHCl_3$), m/e 442 (M^+), Rf (SiO_2, 7 : 3 C_6H_6 - EtAc) 0.65 (visuallized by baking with H_2SO_4). (Found: C, 81.3; H, 11.4, Calc. for $C_{30}H_{50}O_2$: C, 81.4; H, 11.4%). Diacetate m.p. 210-211° from HOAc - Ac_2O (lit.[118] 220°) (Found: C, 77.3; H, 10.3. Calc. for $C_{34}H_{54}O_4$: C, 77.5; H, 10.3%).

Extract B.

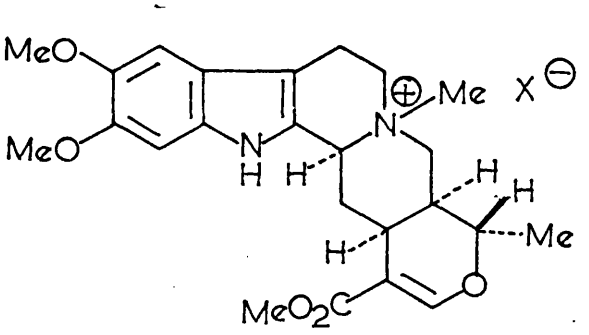
The methanol was evaporated in vacuo and the residual deep red gum (~35g) dissolved in water (1l) and filtered. Insolubles (1.2g) consisted of triterpenoids previously identified and were discarded. After washing with $CHCl_3$ (2 x 200ml) the aqueous extract was chromatographed over cellulose powder (Whatmann CF11, 800g), eluting with ethyl acetate, iso-propanol and water mixtures. The progress of development was monitored by paper chromatography (descending development) employing the Partridge solvent system (upper phase of 4:1:5 nBuOH, HOAc, H_2O). Although chromatograms lacked definition and considerable 'streaking' was evident, it was possible to divide the eluents into five major fractions B_1 (30% iso PrOH-EtAc, 225ml), B_2 (80-100% iso-PrOH-EtAc, 2850ml), B_3 (5% H_2O-iso-PrOH, 700ml), B_4 (~ 20% H_2O-iso-PrOH, 1000ml) and B_5 (H_2O, 2400 ml).

Each of these fractions contained several components and overlapped severely with other fractions. However, an attempt was made to obtain pure compounds by preparative paper chromatography (Whatmann 3MM, 'Partridge' solvent system). Continuous extraction of the bands

produced (fluorescing various shades of blue under UV light) with methanol furnished several gummy residues which could not be induced to crystallize.

SPECTRA

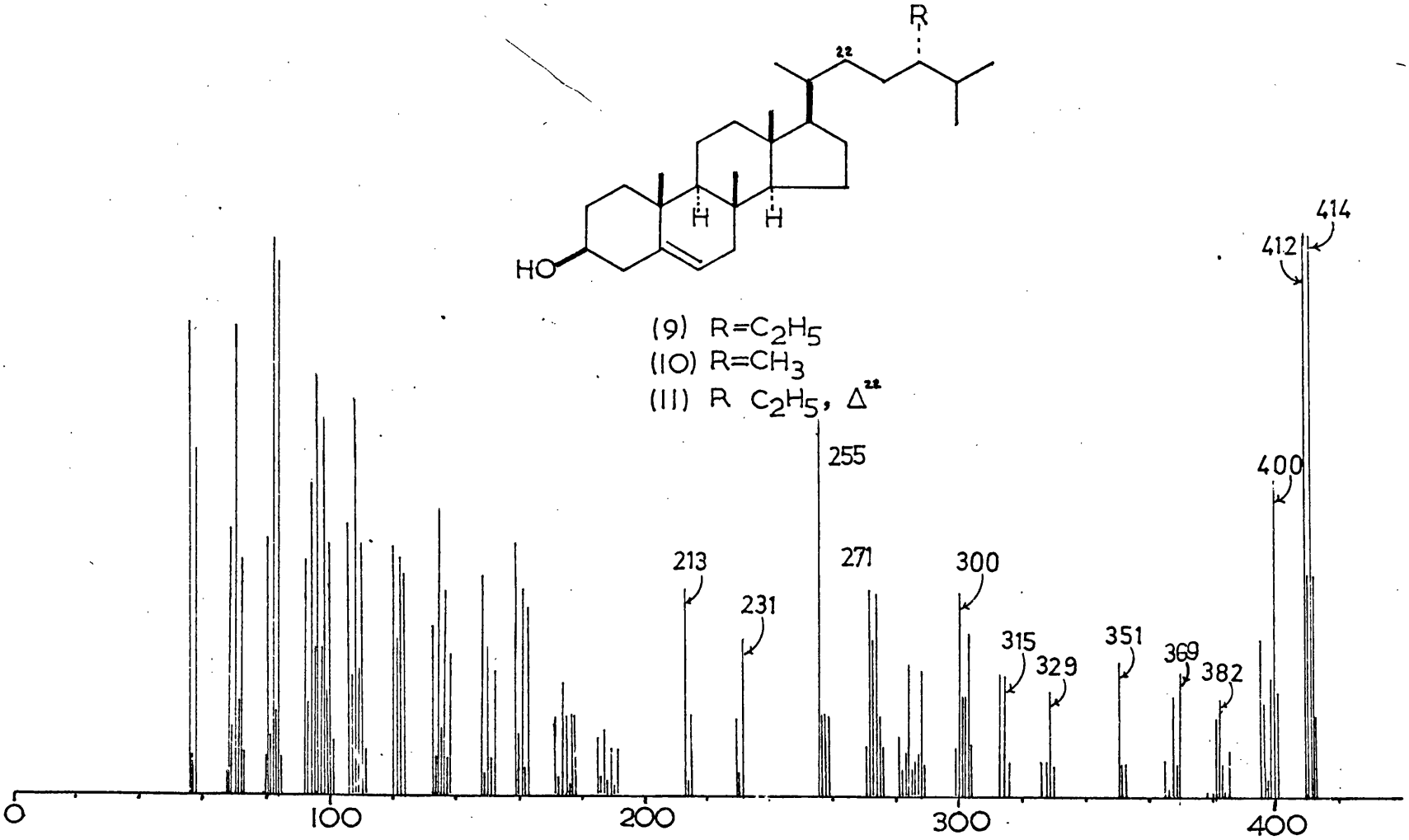
Fig. 2


MeO
MeO
Me
X
N
H
H
H
H
H
Me
MeO2C
O

(13)

412
216
94
96
50
397
122
158
174
230
250
271
283
311
257
325
353
381
426
0
m/e
100
200
300
400

Fig. 3


R
22
H
H
HO
(9) R=C2H5
(10) R=CH3
(11) R C2H5, Δ22
414
412
400
255
271
213
231
300
315
329
351
369
382
0
100
200
300
400

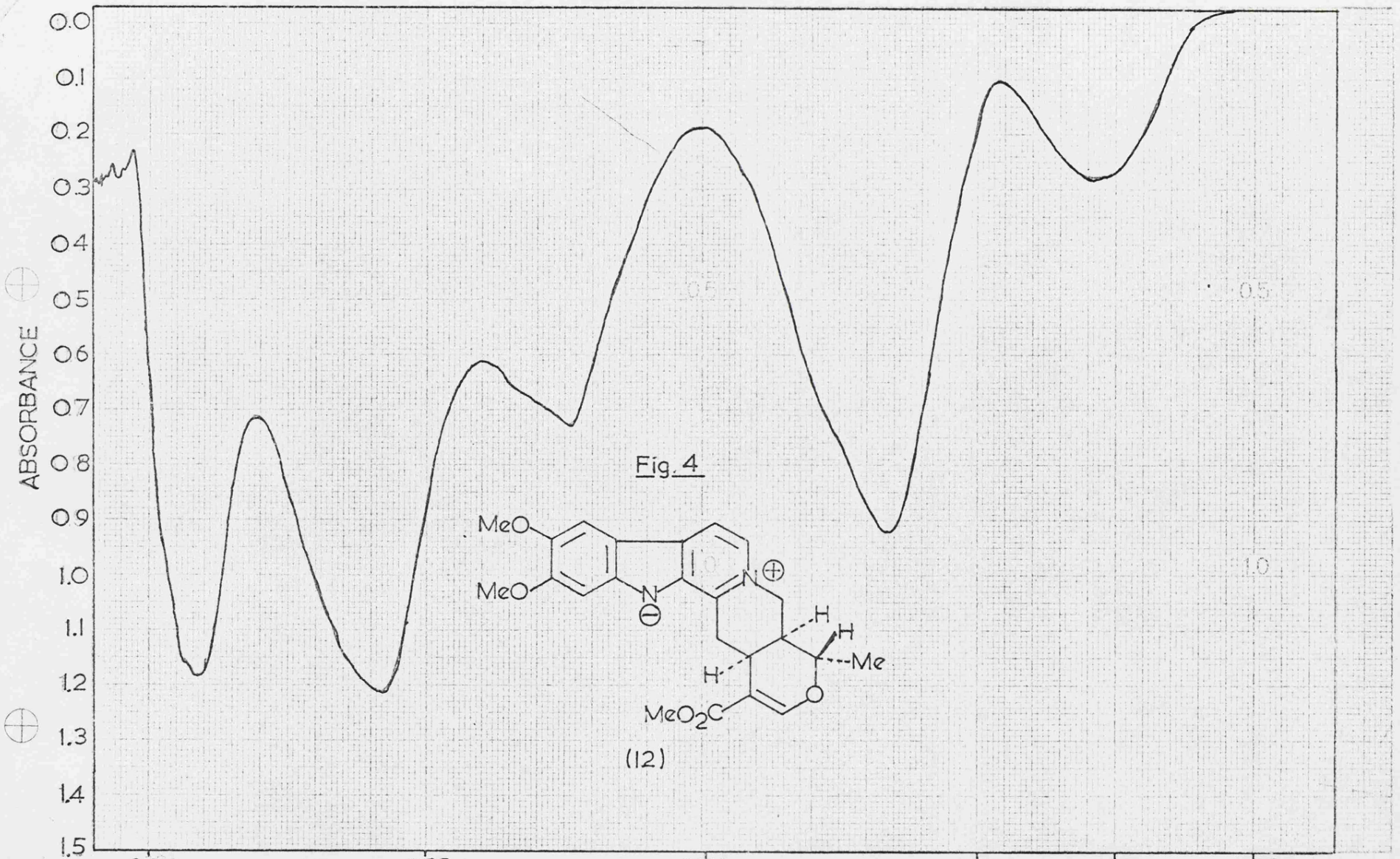

ABSORBANCE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
200
250
300
350
400
450
WAVELENGTH (nm)
MeO
MeO
N
H
H
Me
H
MeO2C
O

Fig. 4

(12)

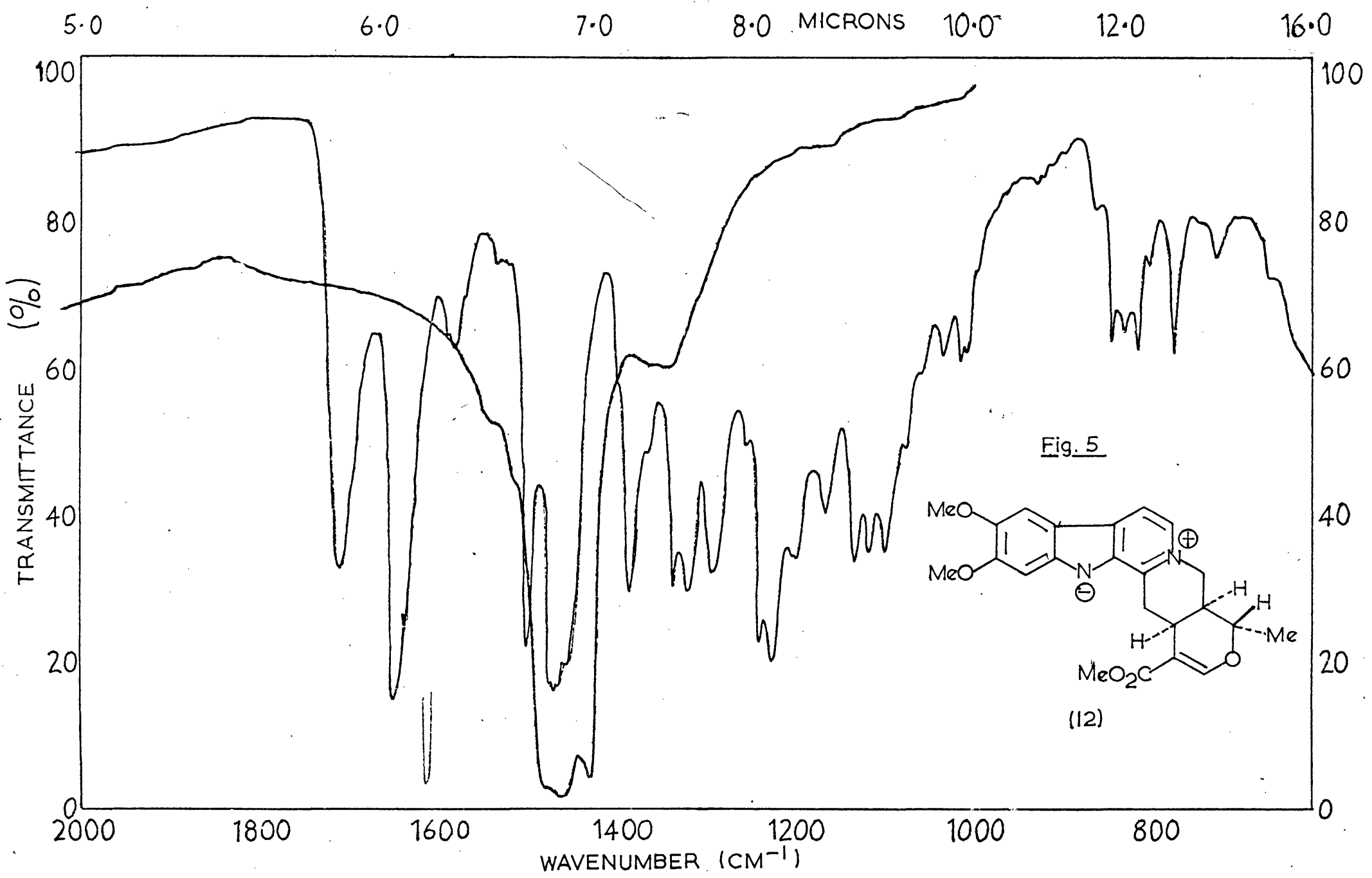
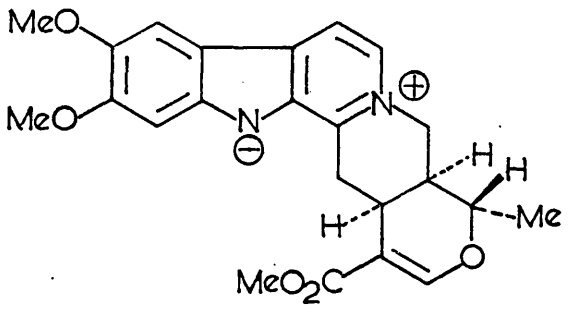

5·0
6·0
7·0
8·0
MICRONS
10·0
12·0
16·0
100
80
60
40
20
0
TRANSMITTANCE (%)
2000
1800
1600
1400
1200
1000
800
WAVENUMBER (CM⁻¹)
MeO
MeO
N
H
H
Me
H
MeO₂C
O

Fig. 5

(12)

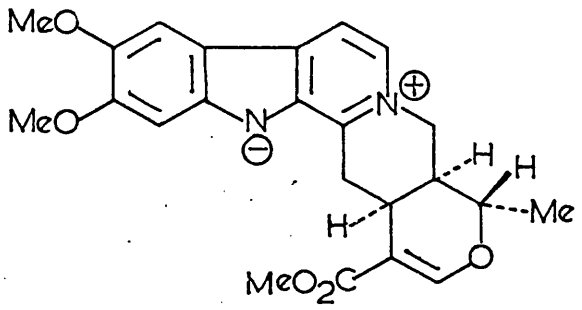
Fig. 6


MeO
MeO
N
N
H
H
Me
H
O
MeO2C

(12)

408 M+
393
267
204
153
179
242
257
281
307
321
349
365
377
0
m/e
100
200
300
400

Fig. 7



(12)

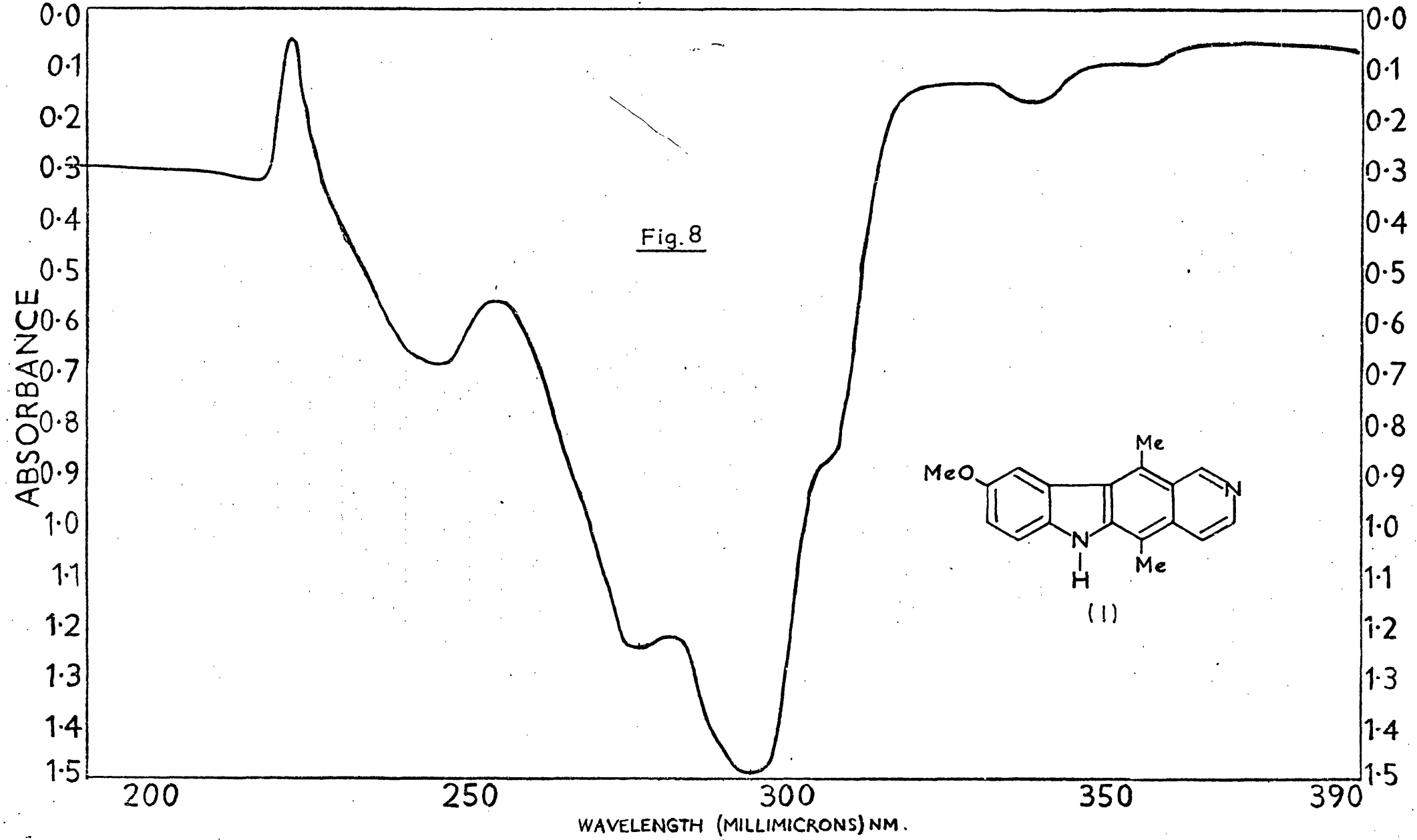

0·0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2
1·3
1·4
1·5
ABSORBANCE
200
250
300
350
390
WAVELENGTH (MILLIMICRONS) NM.

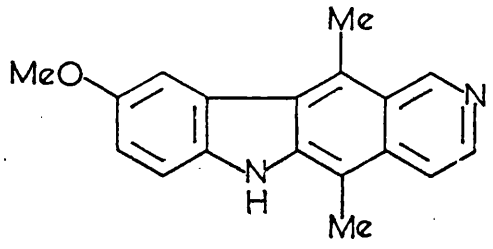
Fig. 8

Me
MeO
N
N
H
Me

(I)

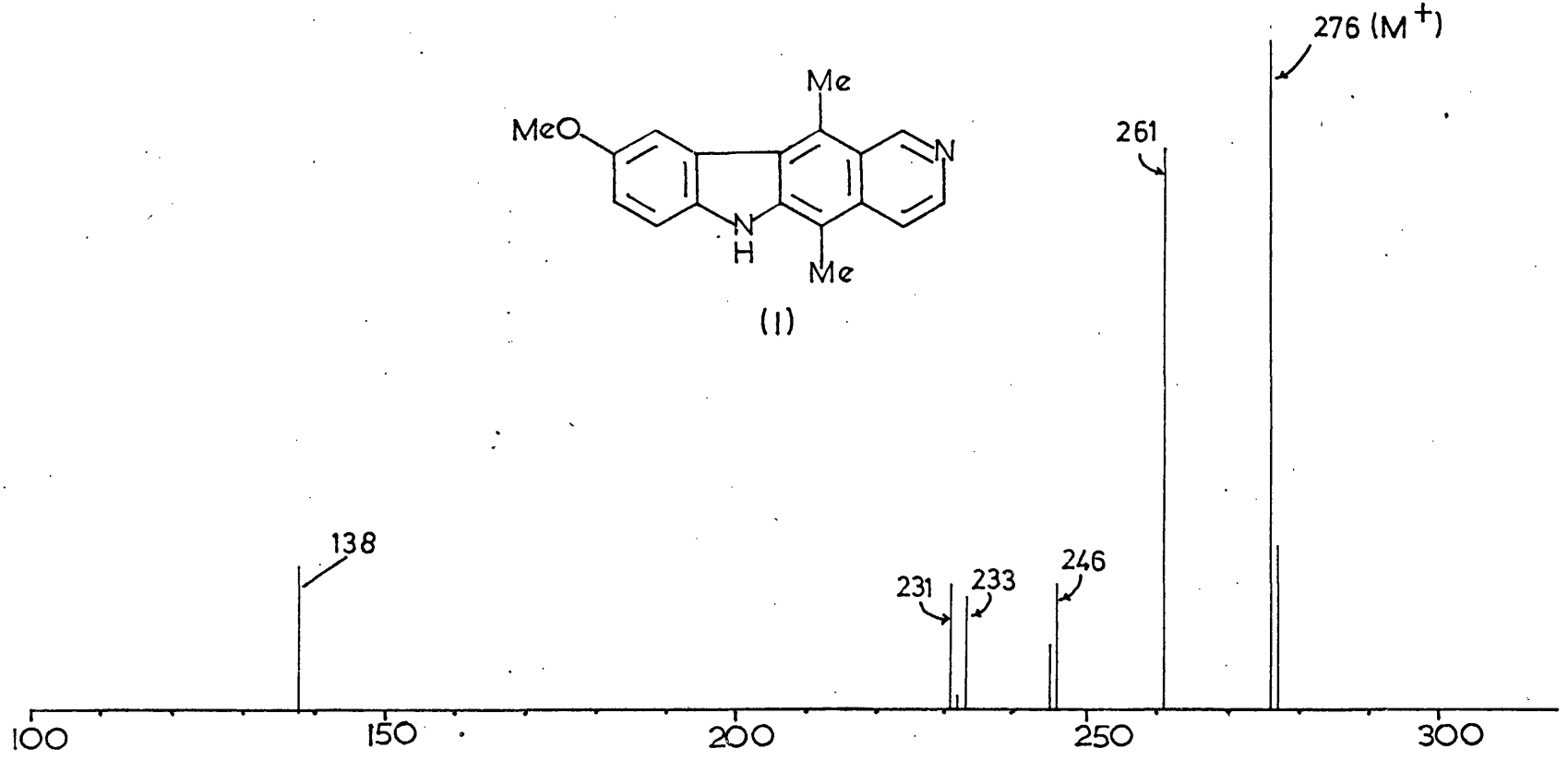
Fig. 9

(Offset 2ppm)



(I)

Fig.10



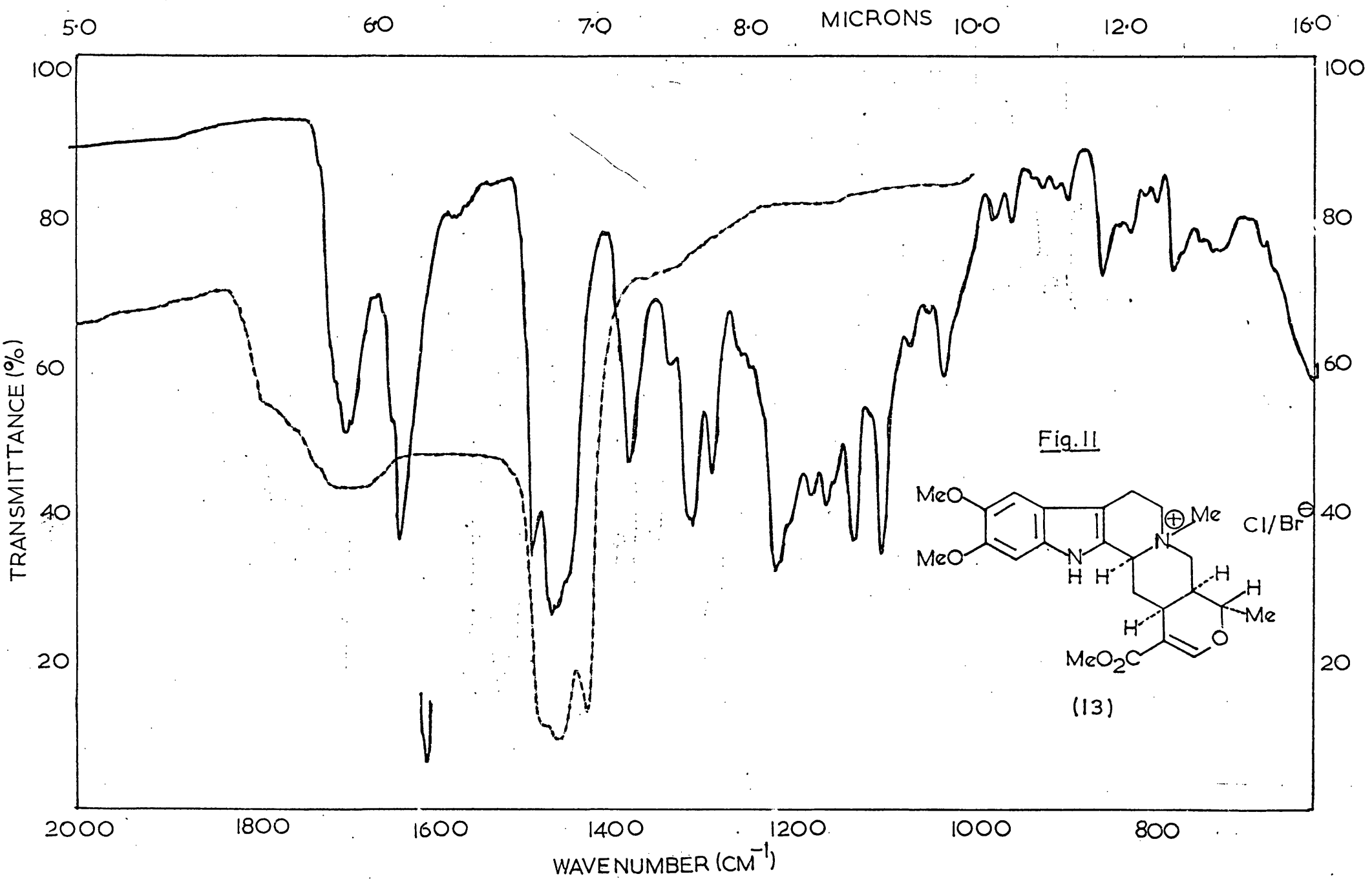
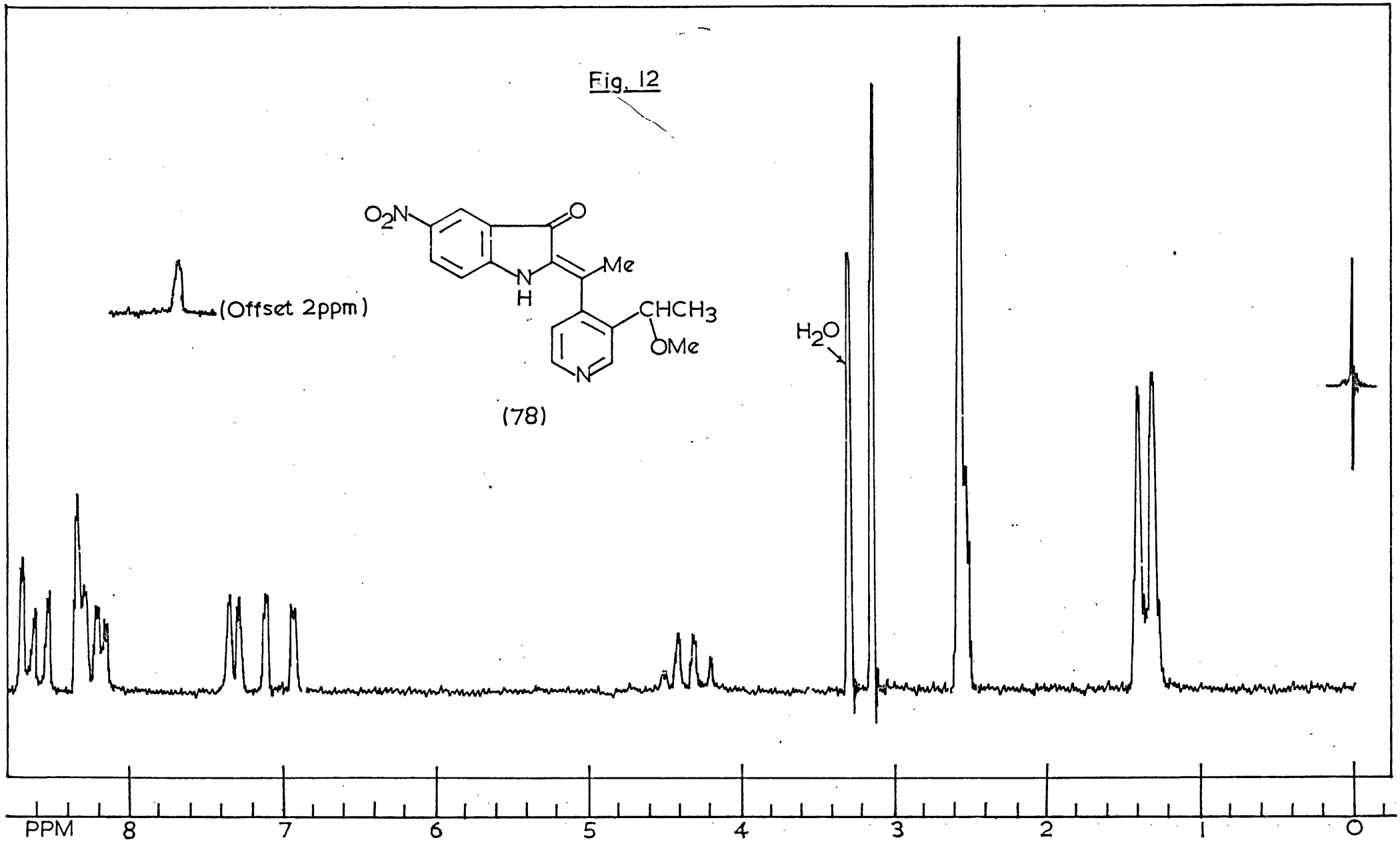

5·0
6·0
7·0
8·0
MICRONS
10·0
12·0
16·0
100
80
60
40
20
TRANSMITTANCE (%)
2000
1800
1600
1400
1200
1000
800
WAVE NUMBER (CM⁻¹)
MeO
MeO
Me
Cl/Br
N
H
H
H
H
H
Me
O
MeO2C

Fig. II

(13)

Fig. 12



(78)

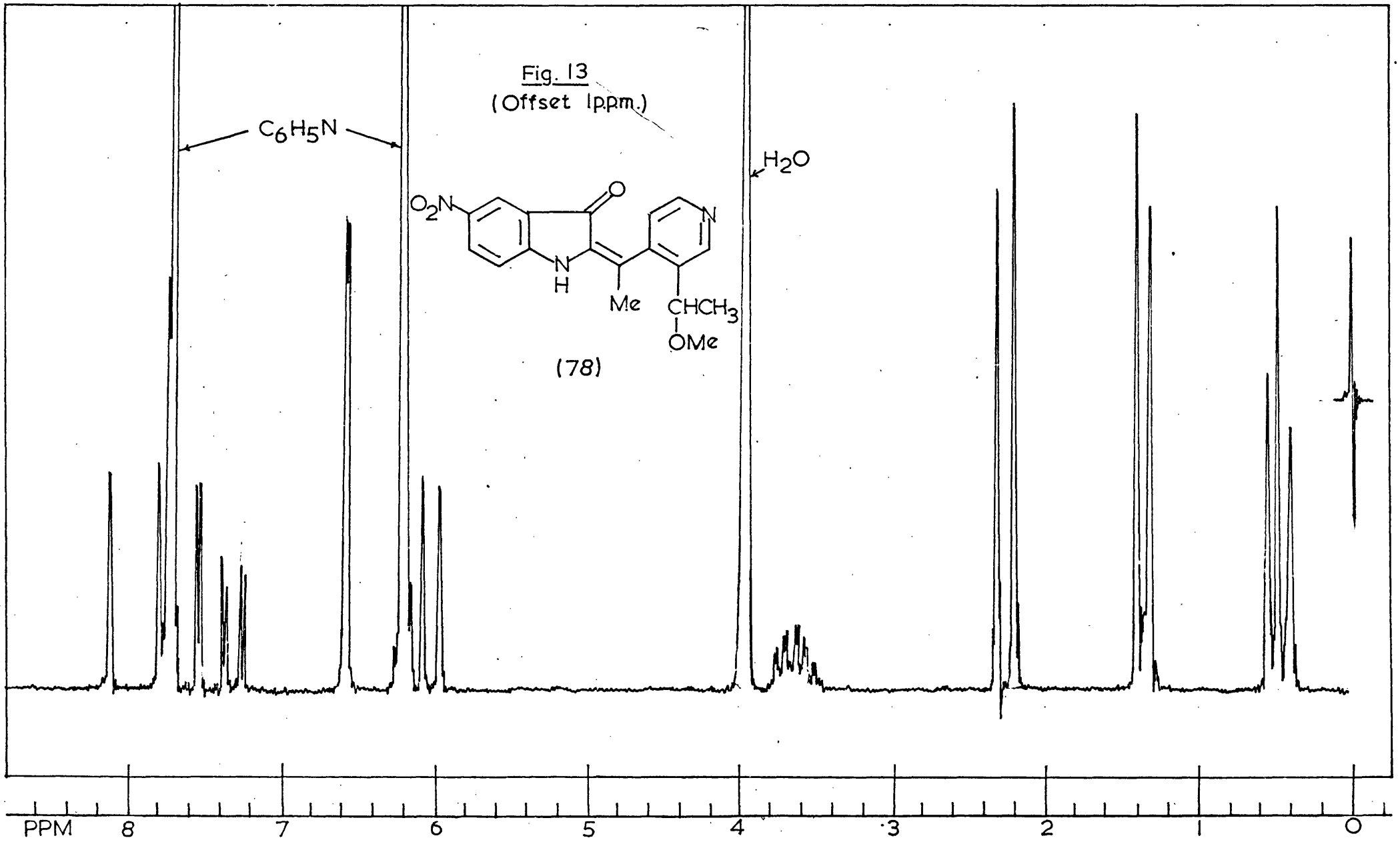
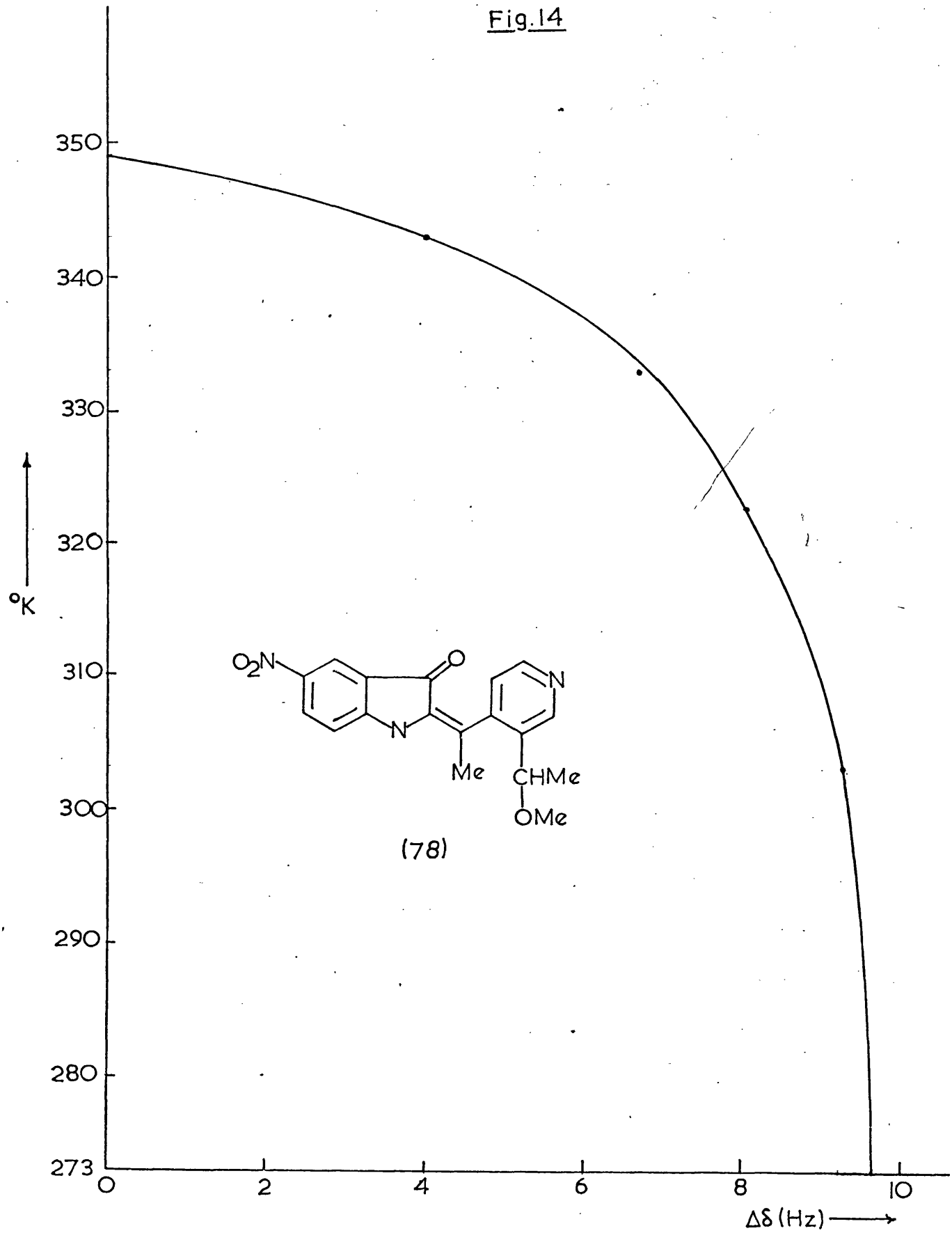


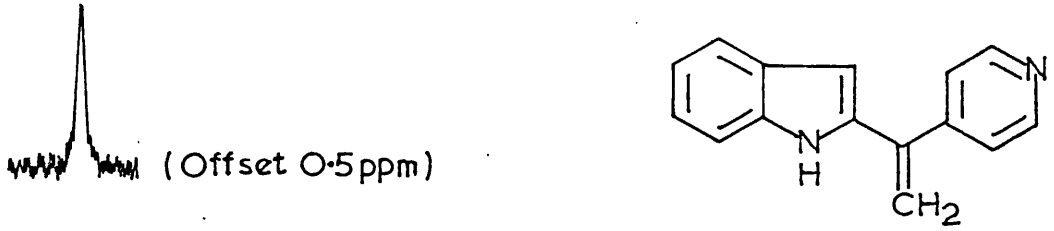
Fig. 13
(Offset 1ppm.)
C_6H_5N
H_2O
O_2N
O
N
H
Me
$CHCH_3$
OMe
(78)
PPM
8
7
6
5
4
3
2
1
0

Fig. 14


350
340
330
320
310
300
290
280
273
°K
0
2
4
6
8
10
Δδ (Hz) ⟶
O2N
O
N
N
Me
CHMe
OMe

(78)

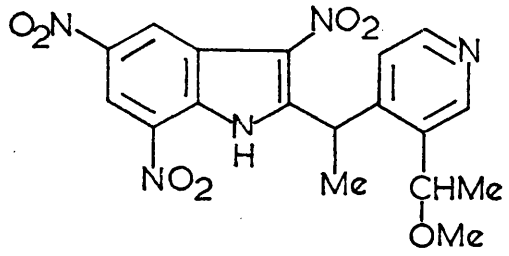
Fig. 15



(81)

(Offset 0·5 ppm)

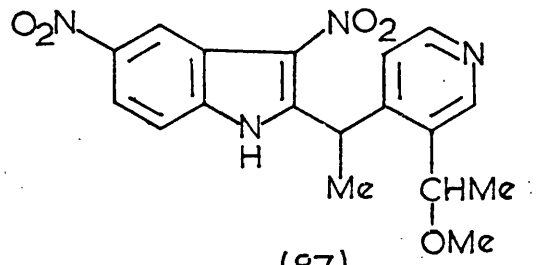
Fig. 16



(86)

Fig. 17

(OFfset 1ppm)



(87).

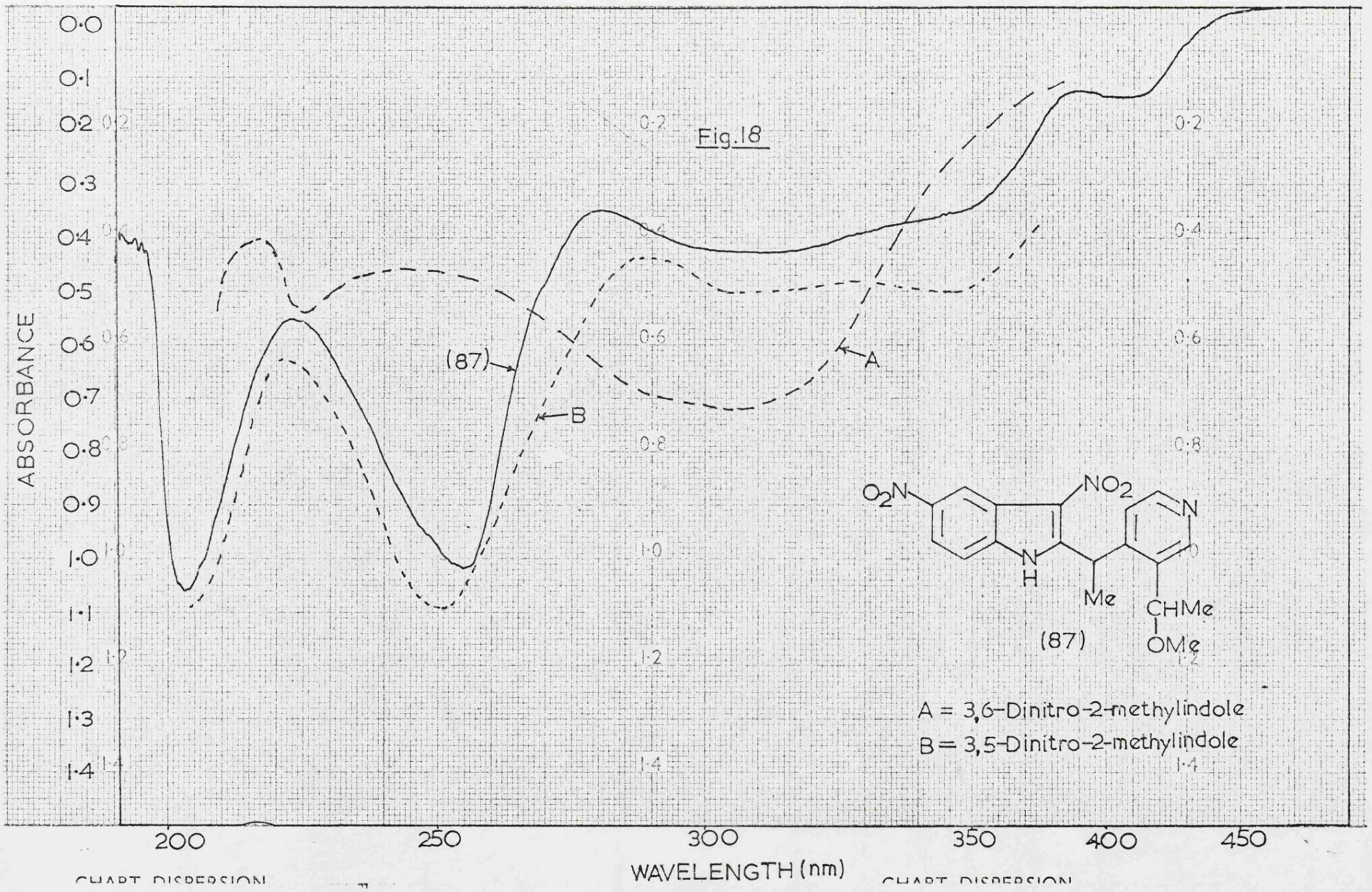
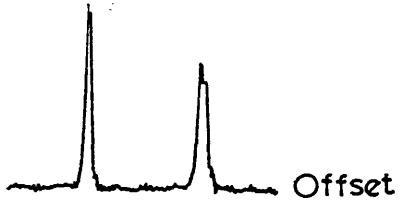
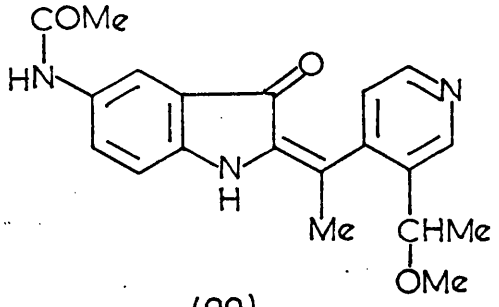


Fig.18
ABSORBANCE
0·0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2
1·3
1·4
200
250
300
350
400
450
WAVELENGTH (nm)
(87)
A
B
A = 3,6-Dinitro-2-methylindole
B = 3,5-Dinitro-2-methylindole
(87)

Fig. 19

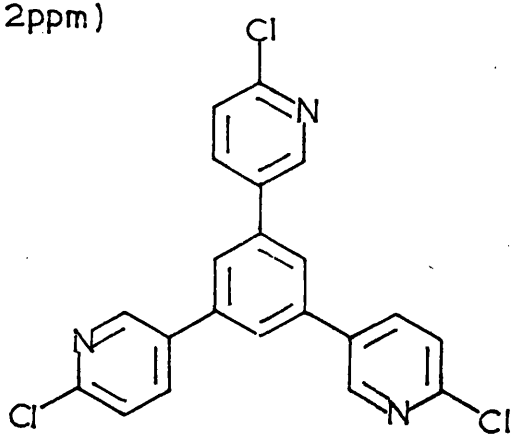




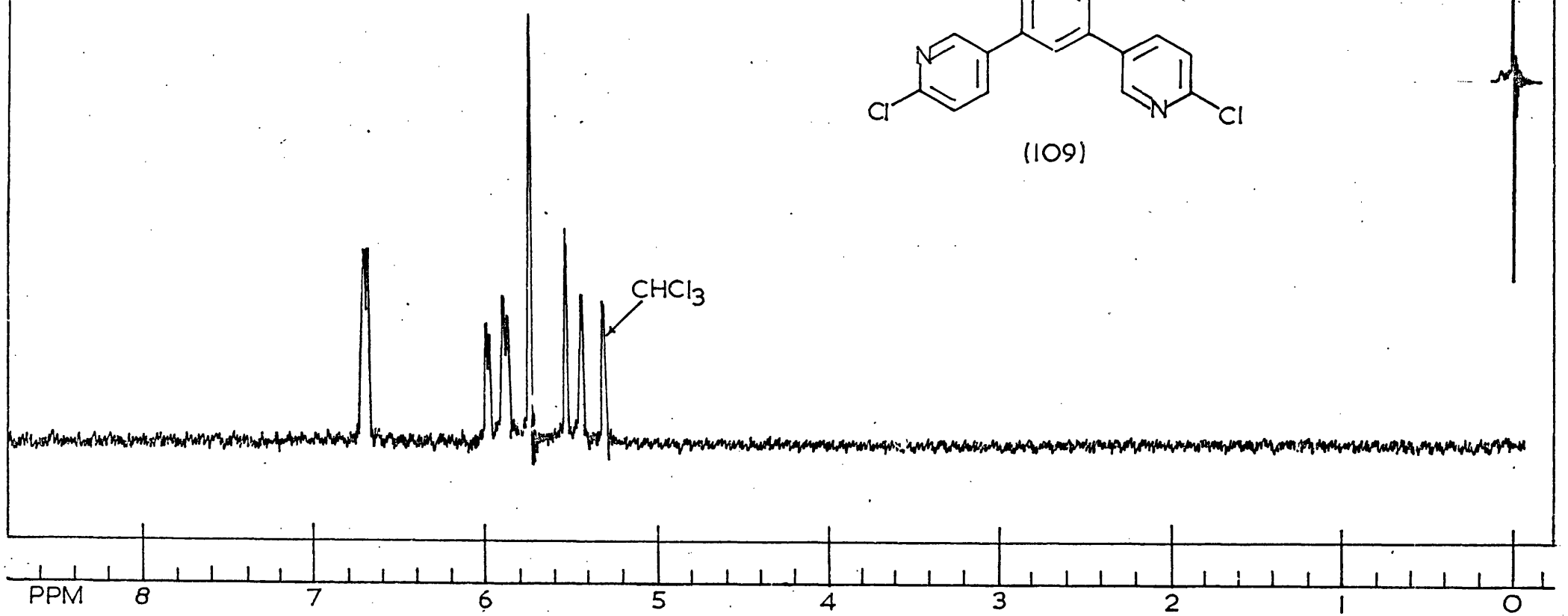
(99)

Fig. 20

(Offset 2ppm)



(109)



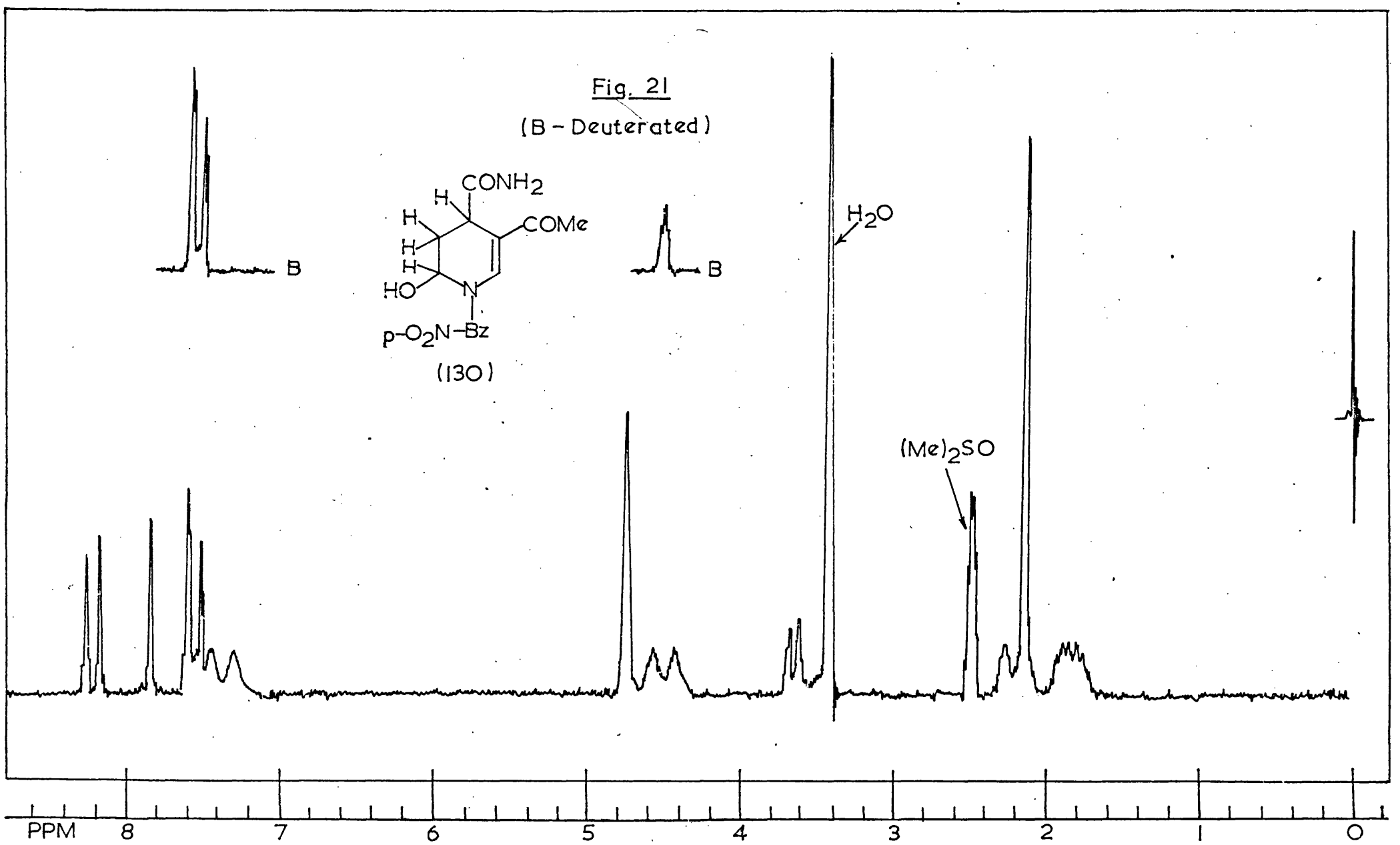
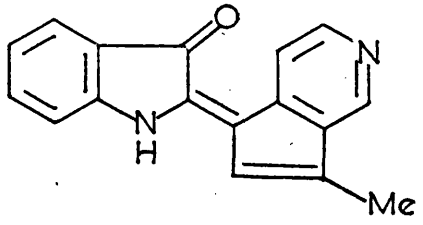


Fig. 21
(B - Deuterated)
CONH2
H
H
H
H
COMe
HO
N
p-O2N-Bz
(130)
B
B
H2O
(Me)2SO
PPM
8
7
6
5
4
3
2
1
0

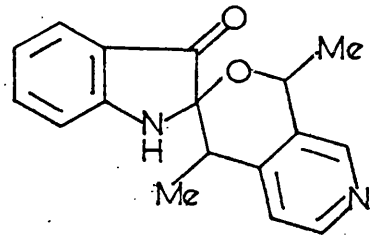
Fig. 22

(Offset 1 p.p.m.)



(146)

Fig. 23

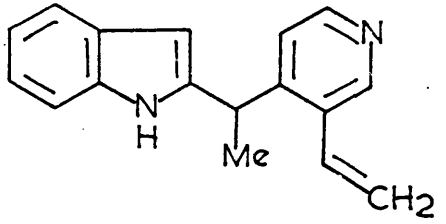

O
Me
O
N
H
Me
N

(157)

(Offset 0·4p.p.m., deuterated)
H_2O
$(Me)_2SO$
PPM
8
7
6
5
4
3
2
1
0

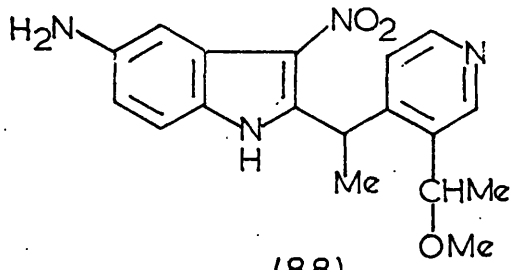
Fig. 24

(Offset 1ppm)



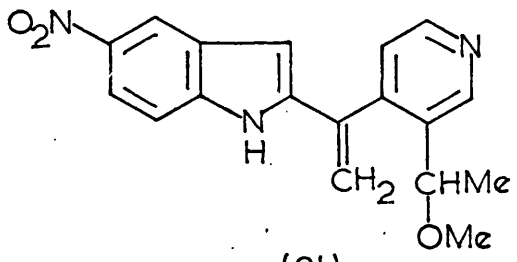
(85)

Fig. 25



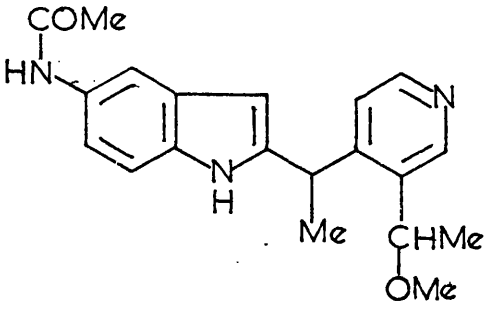
(88)

Fig. 26



(91)

Fig. 27



(101)

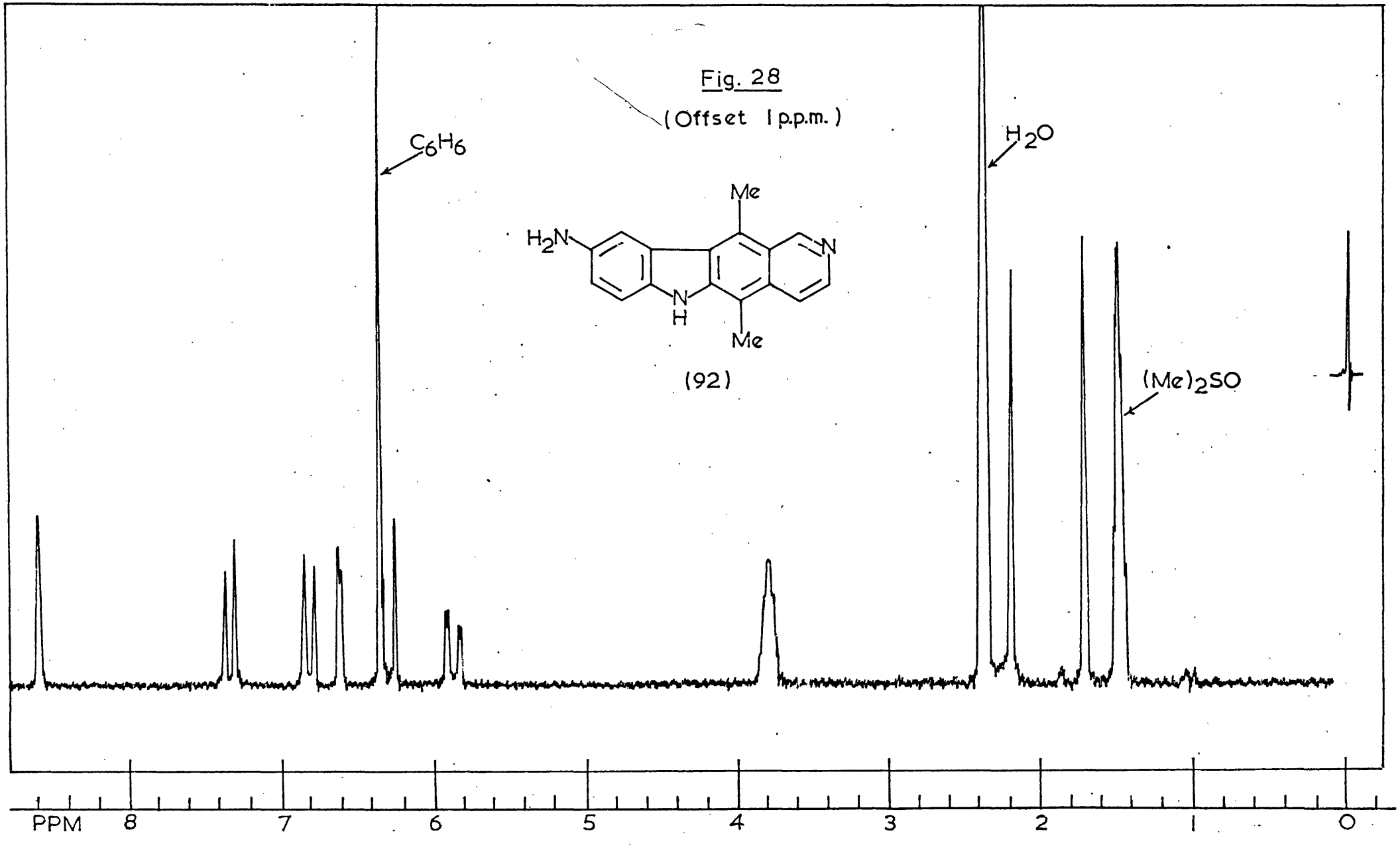
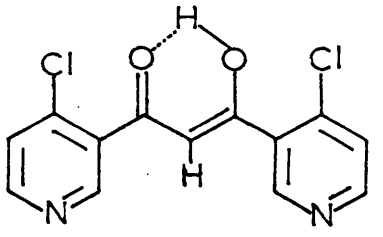


Fig. 28
(Offset 1 p.p.m.)
C_6H_6
H_2O
Me
H_2N
N
N
H
Me
(92)
$(Me)_2SO$
PPM
8
7
6
5
4
3
2
1
0

Fig. 29

(Offset 3ppm)



(112)

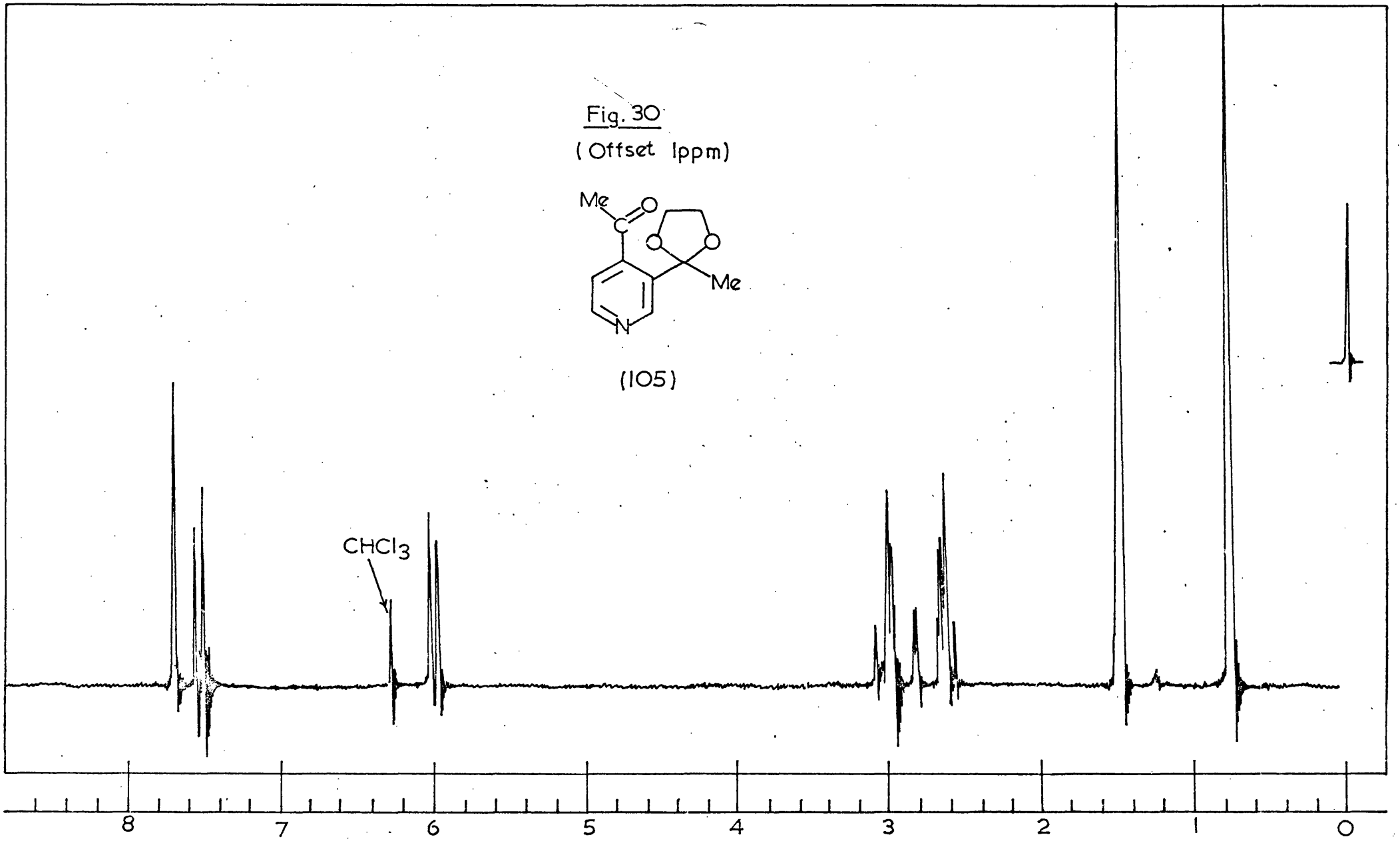
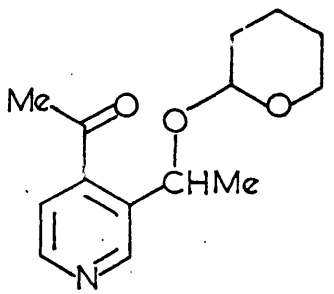


Fig. 30
(Offset 1ppm)
Me
O
Me
N
(105)
CHCl3
8
7
6
5
4
3
2
1
0

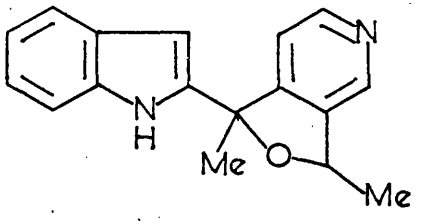
Fig. 31

(Offset 1 p.p.m.)



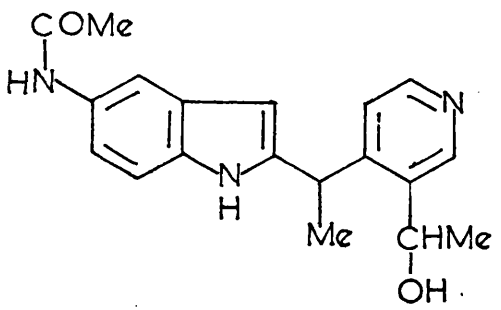
(149)

Fig. 32



(156)

Fig. 33



(155)

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